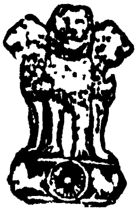

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JOURNAL OF DAIRY SCIENCE

VOLUME XXX JANUARY, 1947 NUMBER 1

THE MALE HORMONE CONTENT OF RUMINANT MANURE[1]

C. W. TURNER

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While the bull testis served as the source of the first extract of male hormone (8, 9) and bull urine was found to be one of the paths of excretion of slight amounts of this hormone (7), it was not until 1942 that Riley and Hammond presented evidence indicating that dairy cattle, during certain physiological states excrete considerable amounts of male hormone by way of the digestive tract in the manure. They noted the precocious development of the comb and wattles of chicks fed dried cow manure as part of the starter ration without stimulation of the gonads. This observation not only indicated the presence of some androgenic hormone in the manure but its oral availability to the chick as well.

The largest content of androgens was observed to be present in the manure of dairy cows in lactation in various stages of pregnancy. Manure from heifers not pregnant produced slight comb development, whereas that from bulls was reported to be without effect.

The secretion of considerable amounts of male hormone by the lactating cow and its elimination in the feces adds significance to the observations of many investigators that androgens stimulate the growth of the mammary glands and may play a rôle in stimulating the lactation process. It seemed of interest, therefore, to study further the extent of elimination of the male hormone by various ruminants preliminary to a detailed study of the excretion rate of this hormone during various physiologic periods.

EXPERIMENTAL[2]

The method of assay of the androgenic hormone in the manure of various species of ruminants has been essentially the same as that described by Riley and Hammond (11). White Plymouth Rock chicks were substituted for Rhode Island Reds. The basal chick starter is the same as used in our

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[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 1009.

[2] I am indebted to Mr. A. J. Olsan for his aid in the collection and drying of the samples and for the care of the chicks during the period of assay of the male hormone.

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previous studies (14) except that the alfalfa meal was eliminated in proportion to the addition of manure up to 10 per cent of the diet. At the end of 4 weeks, the combs were removed even with the surface of the head and weighed. The average comb weight and the comb weight per 100 gm. body weight indicates the extent of androgen stimulation. When the chicks were sexed, the sum of the average weight of the combs of the males and females was divided by two.

Since David (2) showed that the sensitivity of the capon comb to androgen stimulation showed a seasonal variation of as much as 100 per cent from the peak response in winter to the minimum in the summer, the date of each assay is reported. It might be expected that a similar seasonal variation would occur in our assay method since a seasonal rhythm in thyroid secretion rate of chicks has been observed (10) under the same environmental conditions.

The urine-free manure was collected daily, placed in a thin layer in an electric drying oven, and heated to dryness at a temperature of 45° C. or slightly less, except where especially indicated. Usually about 48 hours were required to dry this material. The manure was then ground in a mill to the consistency of alfalfa meal. The starter ration containing the dried cow manure in amounts up to 20 per cent was eaten without apparent discrimination by the chicks.

Unless otherwise indicated, the manure was collected from the same group of lactating cows is free of urine, straw and extraneous material as possible.

EXPERIMENTAL RESULTS

I. *Effect of the temperature of drying on male hormone in cow manure.* In the original study of Riley and Hammond (11) and in later publications (4) it was reported that drying cow manure at a temperature of 80° C. caused the total destruction of the male hormone. To determine the effect of the temperature of drying of cow manure upon the male hormone content under our conditions, samples of cow manure were dried at 45°, 55°, 65°, 75°, and 85° C. in a Freas electric oven. At the lower temperatures about 48 hours were required. At the higher temperatures drying was faster, requiring only 24 hours.

The manure was then ground and fed to groups of about 20 chicks as 10 per cent of the chick starter ration. It will be seen in table 1 that as the temperature of drying manure increased there was a gradual inactivation of the male hormone present. At temperatures above 75° C. all hormone was destroyed since the comb weights were down to control levels.

From these data it appears that the slow drying of cow manure at temperatures above 45° C. for 24 hours or more inactivates increasing amounts of the male hormone present up to about 75° C. It is possible that even a lower temperature of drying would preserve even larger amounts of the hormone, although the drying time would be increased. These data do not answer the question whether higher drying temperatures might not be employed successfully if the time of drying could be reduced to a minimum.

II. *The androgen content of manure of cattle, goats, and sheep.* The following comparisons are based upon the inclusion of 10 per cent of the manure in the chick starter ration. In each case the manure was dried at 45° C. The average weight of the combs of the control White Plymouth Rock chicks at 4 weeks of age varied from 47.8 mg. to a high of 91.0 mg., with an average of 61.3 mg. for 51 chicks. When chicks were fed manure from lactating cows, the combs were much enlarged and bright red in color. The combs of 40 chicks averaged 225.2 mg. in weight—almost a 4-fold increase over the control chicks' combs. This average comb weight is quite similar to that reported by Riley and Hammond (11) for chicks fed manure from lactating cows (237.1 mg.).

TABLE 1

Effect of drying temperature upon male hormone content of cow manure fed at 10% level

(Experiment started, Feb. 14, 1946)

Temperature of drying for 24 to 48 hours	No. of chicks	Average body weight at 28 days	Average comb weight	Comb weight / 100 gm. body weight
°C.		*gm.*	*mg.*	
45	20	259.0	249.2	96.2
55	20	227.6	178.9	78.6
65	21	231.5	76.3	32.9
75	21	232.6	31.2	13.4
85	21	233.5	35.0	14.6

In a preliminary test of mixed bull manure, the average comb weight of the chicks indicated the presence of about one-fourth as much male hormone as did the manure from lactating cows. In a further test of the manure of three Holstein-Friesian bulls varying in age from 2 to 16 years, only slight stimulation was observed.

From the data presented, it would appear that lactating goats are not similar to lactating cows in excreting large amounts of male hormone in their feces. At least, if it is excreted, it is not orally available to chicks. The sample from the pregnant, not lactating, group showed the greatest effect upon the comb. Some male hormone is excreted by the buck since the response of the comb was about double that of the controls and was greater than that shown by the bulls.

Samples from a single, non-pregnant ewe at two periods showed slight responses comparable to that of the non-pregnant goats.

From these observations, it would appear that the lactating cow eliminates a considerable amount of an androgen or androgens which are orally available to the chick. Lactating goats and other ruminant animals both male and female appear to excrete only slight amounts by this route.

III. *Androgen in manure from lactating cows compared with methyl testosterone administered orally.* Riley and Hammond (11) fed 18 mg. of testosterone acetate per kg. of feed in one lot and 18 mg. testosterone acetate + 3.6 mg. of estrone to a second lot of chicks. The comb weights of the two groups were essentially the same. On the basis of equal numbers of the two sexes, the average comb weight of 48 chicks was 649.7 mg. Since the average comb weight of these chicks was far above the comb weight of chicks fed 10 per cent manure, it seemed desirable to determine the amount of synthetic male hormone administered orally equivalent to the amount of male hormone present in the manure of lactating cows.

TABLE 2

The androgen content of dry manure of cattle, goats and sheep
(Fed as 10% of the chick ration)

Type of manure	No. of chicks	Average body weight at 28 days	Average comb weight	Comb weight / 100 gm. body wt.	Date of start of experiment
		gm.	*mg.*		
Control feed	10	305.1	56.3	18.4	Nov.
"	10	227.7	60.8	26.7	Dec.
"	11	307.4	91.0	29.6	Dec.
"	20	223.1	47.8	21.5	May
Cow, lactating	10	257.1	172.6	67.1	Oct.
"	10	296.6	229.8	77.4	Dec.
"	20	259.0	249.2	96.2	Feb.
Bull, mixed	8	230.6	119.5	51.8	Dec.
H-Bull—16 years	20	261.2	62.7	23.9	May
H-Bull— 2 years	19	219.4	59.3	26.7	May
H-Bull— 6 years	20	247.1	73.6	29.5	May
Goat, not pregnant	21	272.0	77.4	29.3	May
Goat, pregnant non-lactating	10	248.1	134.9	54.3	Oct.
Goat, lactating	21	256.9	76.5	29.2	May
Buck	19	223.0	95.2	41.1	May
Sheep, not pregnant	10	307.0	97.5	31.7	Oct.
"	20	221.3	62.9	28.4	May

In a preliminary experiment, groups of about 20 chicks each were fed from 0.75 mg. to 6.0 mg. of methyl testosterone[3] per kg. of feed. As will be observed in table 3, this dosage range was too low to compare with varying amounts of cow manure added to the ration, so in a second experiment the amount of hormone was increased progressively from 10.0 mg. to 40.0 mg. per kg. of feed. There was a regular increase in average comb weight of the chicks up to 30.0 mg. per kg. of feed. When this latter amount was fed dissolved in soybean oil, the comb response was slightly reduced.

[3] Kindly supplied by Dr. E. Schwenk of Schering Corp., Bloomfield, N. J.

For comparison with these data, graded amounts of cow manure were added to the chick ration to cover the same range of average comb response. It will be seen that over the range of 50 gm. to 200 gm. cow manure per kg. feed, the average comb weight compares with the effect of 10 to 30 mg. of methyl testosterone per kg. of feed. In other words, 100 gm. of dry cow manure contains the oral equivalent (in chicks) of 20 mg. of methyl testosterone or 5 kg. of cow manure contains the oral equivalent of 1 gm. of methyl testosterone.

Since 18 mg. of testosterone acetate per kg. feed produced combs weighing 649.7 mg. (11) whereas 20 mg. methyl testosterone per kg. of feed increased combs to an average of 225.2 mg., it would appear that the methyl derivative of testosterone is less effective orally in fowls than the acetate.

TABLE 3

Comparison of comb-stimulating properties of dried cow manure and methyl testosterone administered orally

Material per kg. feed	No. of chicks	Average body weight at 28 days	Average comb weight	Comb weight / 100 gm. body wt.	Date of experiment
		gm.	*mg.*		
Methyl testosterone					
0.75 mg.	21	250.7	51.0	20.3	Jan.
1.50 mg.	19	268.2	88.0	32.8	Jan.
1.50 mg. (in oil)	16	253.4	56.5	22.2	Jan.
3.00 mg.	22	264.0	98.1	37.1	Jan.
6.00 mg.	17	261.5	84.6	32.3	Jan.
10.00 mg.	20	239.3	161.4	67.4	Feb.
20.00 mg.	19	208.2	233.8	112.3	Feb.
30.00 mg.	21	234.8	348.2	148.3	Feb.
30.00 mg. (in oil)	21	234.6	321.3	136.9	Feb.
40.00 mg.	21	226.9	196.7	86.6	Feb.
Cow manure					
25.0 gm. (2.5%)	10	319.5	113.1	35.3	Dec.
50.0 gm. (5.0%)	9	298.6	155.0	51.9	Dec.
100.0 gm. (10.0%)	40		225.2		(see table 2)
200.0 gm. (20.0%)	20	264.5	386.4	146.0	Oct.–Dec.

IV. *Source of the androgen in cow manure.* In the fowl, part if not all of the male hormone produced by laying hens which stimulates the growth of the comb is believed to be secreted by the medullary part of the ovary (3). In the mouse, the secretion of male hormone by the ovaries is stimulated when ovarian grafts are made into the ear (5). Burrill and Greene (1) present evidence indicating the secretion of male hormone in the pregnant and lactating rat. In man, both normal male and female urine contains considerable amounts of both estrogens and androgens.

In cattle, the male hormone was observed to be excreted in small amounts in the urine of the bull (7). Little has been reported on androgn content of cow urine. The observations presented in this paper indicating the presence of considerable amounts of androgens in the feces of lactating cattle raise the question as to the source of the male hormone and the part of the digestive tract where the androgen is passed into the contents of the tract. In addition to the ovaries, it is well established that the adrenal glands may under certain conditions secrete male hormone.

As might be expected, it has been reported that androgens are not present in dried rumen contents (4). From our knowledge of the metabolism of other hormones, the liver might be expected to play a rôle in the elimination of androgen by way of the bile from either the ovaries or adrenals, although it is possible that it might be excreted directly by the cells lining the digestive tract.

Since milking cows were not available, the entire digestive tracts of two Shorthorn type cows were obtained from a local packing house for our preliminary study. The livers and bile were also retained. The rumen contents were each sampled and dried. The contents of the omasum, small intestine and large intestine of each cow were combined and dried. The livers were dried and ground and the bile mixed with the chick feed and dried. The average weight of the combs of the chicks indicated insufficient androgen present to indicate the point in the digestive tract where the products of androgen metabolism were discharged into the digestive tract.

While the combs of the chicks fed graded amounts of liver were almost

TABLE 4

Source of androgen

(20 chicks in each lot)

Material	Amount fed	Average body wt. chicks at 28 days	Average comb weight	Comb weight / 100 gm. body wt.	Date of experiment
		gm.	*mg.*		
Cattle					
Rumen content					
Cow I	10%	234.7	72.8	31.0	April
Cow II	10%	217.7	48.0	22.0	April
Omasum content, mixed	10%	233.8	40.9	17.4	April
Small intestine, mixed	355 gm. in 10 kg.	220.5	49.3	22.3	April
Large intestine, mixed	10%	225.2	38.6	17.1	April
Dried liver	5%	275.1	63.5	23.0	April
" "	10%	295.6	74.3	25.1	April
" "	15%	298.0	75.9	25.4	April
Bile	470 ml.*	276.7	55.2	19.9	April
Goat rumen	10%	202.1	45.0	22.2	Dec.
" urine	5876 cc. dried in 10 kg. feed	233.3	52.3	22.4	April

* Since no more cattle bile could be obtained, 735 ml. of hog bile was added.

double the control comb weight, the fact that their size was not closely graded in proportion to liver dosage raises a question as to the significance of the greater comb weight.

This preliminary work clearly indicates the necessity of using lactating cows in such a study and to have evidence that the cows being used are discharging normal amounts of androgens in their manure.

DISCUSSION

The discovery of the excretion of considerable amounts of androgenic hormone in the feces of the lactating dairy cow is of considerable interest from several points of view. The use of dried cow manure in the ration of chickens and swine as a rich source of B-vitamins with nutritive value comparable to an equivalent amount of alfalfa meal raises the question of the desirability of utilizing this by-product of dairy farming as a feeding stuff rather than as a fertilizer.

In addition to its nutritive value, dried cow manure is an economical source of an orally available androgenic hormone. It has been shown in rats that the injection of androgens in suitable amounts stimulates the rate of growth (12–13). It is also recognized that this hormone favorably influences nitrogen metabolism (6). Work in this laboratory indicates that the feeding of dried cow manure at suitable levels to chicks from hatching time until sexual maturity stimulates the growth of the pullets especially, and the age of the onset of egg production was reduced.

The rôle of the male hormone in lactating dairy cows is especially intriguing. Evidence is constantly increasing indicating that both male and female sex hormones are being produced by the endocrine glands of both males and females in considerable amounts. It has been demonstrated that certain androgenic hormones stimulate the growth of the mammary glands experimentally. However, up to the present time the male hormone has not been suggested as playing a dynamic rôle in either the normal growth of the cow's udder or in the initiation or maintenance of milk secretion. This problem deserves serious consideration in the light of the observations reported.

SUMMARY

The male hormone (androgen) content of ruminant manure was assayed biologically by feeding it as part of the starter ration to groups of White Plymouth Rock chicks during a period of 28 days. The average comb weight of 40 chicks fed 10 per cent dried manure from lactating cows was 225.2 mg. in comparison to the comb weight of normal chicks of 61.3 mg.

Lactating cow manure dried at temperatures ranging from 45° C. to 85° C. by 10 degree intervals gradually declined in androgen potency. At temperatures of 75° C. and above all biological activity was lost.

The androgen content of the manure of other ruminants including goats

and sheep of both sexes and conditions was either low or absent. The manure of dairy bulls showed indications of only small androgen excretion by that route.

Chicks fed methyl testosterone at the rate of 20 mg. per kg. of feed had combs averaging 233.8 mg. This is comparable to the effect of lactating-cow manure fed at the 10 per cent level or 100 gm. per kg. of feed.

The point of entrance of the androgenic hormone into the digestive tract of the cow has not yet been determined.

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SOME OBSERVATIONS ON NERVE REGENERATION IN THE UDDER[1]

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The theory has been generally accepted that cows "let down" their milk as a result of afferent stimuli which originate in the udder. The most common of these stimuli are nursing by the calf or milking by hand or machine. Ingelbrecht (1), working with rats, claimed that the "letting down" of the milk in the mammary gland was dependent upon an intact nerve supply. When he destroyed the nerves to the posterior mammary glands and covered the anterior glands the rats failed to lactate normally and the young soon died. But when he permitted the young rats to nurse the glands with an intact nerve supply, every gland secreted milk and the rats grew normally.

Ely and Petersen (2) consider this phenomenon due to a nerve-endocrine mechanism rather than to a nervous reflex. They suggest that the afferent impulses produced by milking cause the posterior lobe of the pituitary to release oxytocin. The oxytocin acts upon the smooth muscle fibers surrounding the alveoli in the mammary gland and forces the milk in the cells and lumina of the alveoli into the larger collecting ducts and udder cisterns. It is at this particular moment that a cow is said to have "let down" her milk.

If their theory is correct only the milk in the udder cisterns could be obtained from a cow in the absence of this nerve-endocrine stimulus. Although a cow may be so conditioned to milking that various afferent stimuli cause her to "let down" her milk, the nursing of the calf or the massaging of the udder in milking should be the primary stimuli. Ely and Petersen (2) found that cutting the inguinal nerve on three dry cows had no effect on milk secretion when the cows freshened about two months later. This procedure should have eliminated any direct effect of the nerves on the musculature of the udder, unless nerve-regeneration occurred. But cutting of the inguinal nerve does not interfere with the sensory nerve supply to the skin of the udder.

EXPERIMENTAL

In order to destroy all nerve pathways to the mammary gland, an udder transplant was made on a Holstein calf when she was about six weeks of age. The mammary glands were dissected free of the body except for one exter-

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nal pudic artery which was left intact. The dissected glands were turned around so that the rear teats faced anteriorly, and the udder was then sewed back into place. After the skin lesions had healed a second incision was made on the right side of the glandular area and the external pudic artery which had previously been left intact, was ligated.

The heifer was bred at as early an age as possible. She freshened when less than 22 months of age. This may help to explain why she failed to clean normally and why she was rather slow in coming to her milk. However, she was able to provide sufficient milk for her calf and another calf which nursed her. Although the udder presented a most unusual appearance the day after parturition (fig. 1), its development during pregnancy seemed quite normal. One month after freshening the calves were weighed before and after nursing (twice a day) for three days. By this method of calculation it was found that she was producing 23½ pounds of milk per day, in spite of the fact that the right rear gland (as now attached) gave almost no milk. Bacteriological examination showed that the gland was badly infected with mastitis. Except for this quarter the mammary glands produced an apparently normal secretion. Treatment with penicillin failed to return the diseased gland to the same production as the left rear quarter.

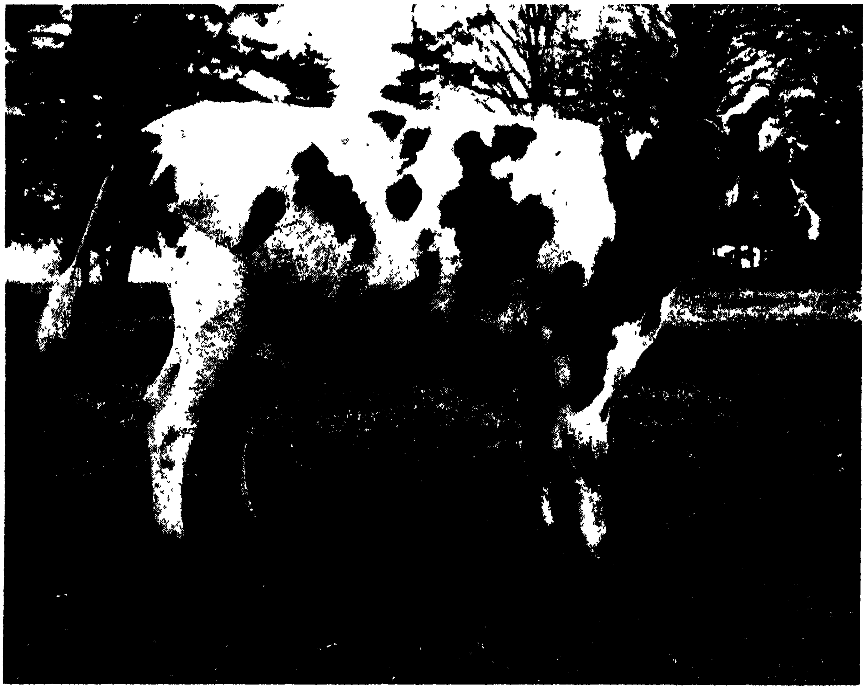

FIG. 1. Cow whose udder was reversed and all nerves and blood vessels ligated.

An electrical current from a secondary coil was used to determine the sensitivity of the udder to external stimuli. Although the left side of the udder did not seem quite as sensitive as the skin above the incision there was no point on the left half of the udder when the electrical current did not cause the cow to jump. The entire right half of the udder was devoid of a sensory nerve supply. The only explanation the author has for this discrepancy in nerve supply between the two halves of the udder is that the regenerating nerves were destroyed on the right side of the udder when the second incision was made. However, if this explanation were valid, it seems strange that the anterior and posterior surfaces of the right half of the udder (where only the one incision was made) would be as devoid of a sensory nerve supply as the area immediately below the second incision. Every effort was made to ligate all of the mammary tissue during the two operations, yet it is conceivable that this was not done. Failure to completely sever all of the nerves seems a more probable explanation, especially since electrical stimuli when applied to the left side of the udder caused the cow to kick with her right foot.

TABLE 1

Milk obtained from transplanted udder

Quarter	Amount of milk obtained in first 4 minutes of milking	Amount of milk obtained in last 2 minutes of milking
	pounds	*pounds*
Right front	1.5*	0.7†
Right rear	Not used because of mastitis	
Left front	3.2	0.4
Left rear	2.8	0.5

* Before any known afferent stimuli had caused the cow to "let down" her milk.

† After the calves have nursed for 2 minutes the glands with an afferent nerve supply (left front and left rear).

Since this cow had never been milked by hand there should have been a minimum amount of interfering conditioned reflexes established. A milking machine with one teat cup was used for these experiments. The cow was locked in her stanchion in a box stall at the time when the calves regularly nursed. Instead of allowing the calves to nurse, the experimenter would curry the cow for a couple of minutes to secure her confidence. The milking machine was then attached as quietly as possible. After four minutes, the milking machine was removed and the calves allowed to nurse all but the gland under observation. When the calves had nursed for approximately two minutes, the milking machine was again attached to the gland under observation and milking was continued for another two minutes. The results are given in table 1.

The results presented in table 1 are too meager to offer conclusive evidence. Certainly the glands which have an intact nerve supply were emptied much more quickly than the one "normal" gland without an afferent nerve supply. The reader may wonder why approximately 1½ pounds of milk were secured from the right front gland before the nursing of the calves provided the essential nerve-endocrine stimulus suggested by Ely and Petersen. Certainly this amount of milk was not already present in the gland and teat cistern. Although no definite readings were made, the milker is positive that a goodly proportion of this milk was obtained during the last half of the four-minute milking period. The only explanation the author can suggest is that the emptying of the udder provided a certain indirect stimulus. Although a half-inch spark to the end of the right front teat elicited no response from the cow, before the four-minute milking interval was completed she manifested a certain awareness to the action of the machine. This was especially noticeable when the milking machine was removed.

SUMMARY

The mammary tissue of a calf was transplanted so as to sever all of the original nerve and vascular supply between the body and mammary glands. Normal development of the udder occurred during pregnancy with the exception of the right half of the udder which had no sensory nerve supply. A simple experiment on the nerve-free gland has helped to confirm the theory of Ely and Petersen in regard to the functioning of a nerve-endocrine mechanism in the "letting down" of the milk.

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PEPTIC-TRYPTIC DIGESTION OF RENNET-CUSTARDS IN VITRO

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The present paper describes further studies in which plain and rennin-treated milks (rennet-custards) were first digested artificially by pepsin-hydrochloric acid mixtures, and then exposed to additional digestion by synthetic mixtures of pancreatin and bile.

The possibility that cow's milk can be made more easily digestible by exposure to enzymes, such as the rennet enzyme, prior to ingestion, has not been adequately explored. Both Doan and Dizikes (2) and Turner (5) have observed that milk previously exposed to tryptic (pancreatin) digestion in vitro for a short period became broken down more quickly than ordinary milk. Actual feeding of infants with enzyme-treated milks has been carried out by Blatt (1), who stated that such treated milk proved easily digestible, well tolerated and well utilized, even by premature infants.

The influence of added rennin upon curd-forming properties and peptic digestion of milk has already been discussed in a previous publication (4). This study showed that when rennet-custards were exposed to conditions of hydrochloric acid and pepsin resembling those in the normal stomach, they underwent protcolysis more rapidly than untreated milks used as controls. However, the degree of proteolysis as measured by the amount of casein made soluble in the whey filtrate was relatively small. Evidently, gastric digestion of milk is only preparatory to the major digestive activity of the small intestine.

PROCEDURE

In the experiments here reported, the artificial coagulation device (3) and the artificial digestion procedures (4) were the same as already described. The rennet-custards were prepared as follows: Lukewarm pasteurized milk was mixed with the rennet preparation undergoing test, and 100-ml. portions of the mixture poured promptly into the rubber bags in the coagulation device. These stood motionless in the water bath at a temperature of 37° C. for 5 minutes, during which time the coagulation into rennet-custards took place. Lukewarm control milks, without rennet, were added to similar bags at this time.

The apparatus was next set in motion. One ml. of a 0.6 per cent pepsin solution containing sufficient N/1 hydrochloric acid to lower the pH to 5.5 was then added to each bag. (The pH of 5.5 reproduces the approximate hydrogen ion concentration at which milk normally coagulates in the child's stomach (6).) After the control milks had coagulated, the apparatus was then agitated for one-half hour. Two ml. of the pepsin solution and sufficient

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additional hydrochloric acid to bring the mixture to the desired pH were next mixed in each bag and the agitation permitted to continue for 2½ hours longer.

The rennet-custards and the control milks were handled in the same way. When rennet tablets[1] were used, the controls had the same amount of water added as did the rennet-custards, omitting the dissolved tablet; when rennet powder[1] was used, all ingredients of the powder, except the rennin itself, were added to the control milks.

The next step was digestion by pancreatin-bile solutions of varying strengths. After exposure of the milk to the pepsin-hydrochloric acid activity for 3 hours, the pH was raised to 7.5 by a strong sodium hydroxide solution, a solution of pancreatin-bile was mixed in, and the digestion continued for 2 hours more. During this period representative samples were removed at 20, 60, and 120 minutes. The digestion experiments were grouped into three series, according to the concentrations of pepsin-hydrochloric acid and pancreatin-bile used (table 1). In the first series, 3 ml. of 0.6 per cent pepsin solution, together with sufficient hydrochloric acid to produce a pH of 2.5, were mixed with 100 ml. milk. In the second series, 1 ml. of 0.6 per cent pepsin solution, together with sufficient hydrochloric acid to produce a pH of 3.5, were added. In the third series, 0.5 ml. of 0.6 per cent pepsin solution, together with sufficient hydrochloric acid to produce a pH of 5.5, were added. Thus, the concentration of pepsin in the second series was one-third, and in the third series, one-sixth of that in the first series.

The pancreatin-bile consisted of a solution of 3 gm. pancreatin powder (U.S.P. XII) plus 3 gm. bile powder (Bacto lactose peptone bile, dehydrated) in 100 ml. water. Of this solution 3 ml. were added to each experimental specimen in the first series, 1.5 ml. to each in the second series, and 0.5 ml. to each in the third series. Enough sodium hydroxide was introduced along with the pancreatin-bile mixture to keep the pH constantly at 7.5, corresponding to the hydrogen ion status of the upper part of the resting small intestine. For purposes of convenience, the three procedures have been designated as "stronger," "intermediate," and "weaker" peptic-tryptic digestion.

The amount of soluble nitrogen in the filtered whey was measured by the macro Kjeldahl method. The amount of soluble nitrogen in the blank test, *i.e.*, in the milk itself prior to any of the digestive manipulations, was sub-

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[1] The rennet preparations used to make rennet-custard were: Household rennet tablets, composed of powdered rennet—2.84% and table salt—97.17% (one 0.55 gm. tablet per 500 ml. milk). Rennet powder, containing salt with added sugar and vanilla flavor, and composed of sugar 98.59%, salt—1.37% and rennin—0.04% (45 gm. of powder per 500 ml. milk). Each preparation contained sufficient rennin to coagulate 500 ml. milk in 2½ minutes at 110° F.

The tablets and powder were secured from Chr. Hansen's Laboratory, Inc., Little Falls, N. Y.

TABLE 1

	Pepsin-HCl mixture	pH	Pancreatin-bile mixture	pH
	cc.		*cc.*	
1. "Stronger" peptic-tryptic digestion	3.0	2.5	3.0	7.5
2. "Intermediate" " "	1.0	3.5	1.5	7.5
3. "Weaker" " "	0.5	5.5	0.5	7.5

tracted, and the difference taken as indicative of the rate of digestion at the end of the subsequent pancreatin-bile digestion. To measure the extent of trypsin digestion of casein, the method of the U. S. Pharmacopoeia (XII) was used. A few ml. of a specially prepared acetic acid-alcohol solution were added to the samples just before filtering. This solution precipitates that fraction of the casein which has become soluble but not yet fully broken down.

RESULTS

The mixtures of pepsin-hydrochloric acid and pancreatin-bile used in these experiments are believed to simulate the conditions prevailing in the stomach and small intestine of children at successive stages of growth. The exact amounts of the several components which would enter into the actual digestion of 100 ml. of milk are not precisely known, of course. The results of this study are to be viewed as comparative rather than absolute; they

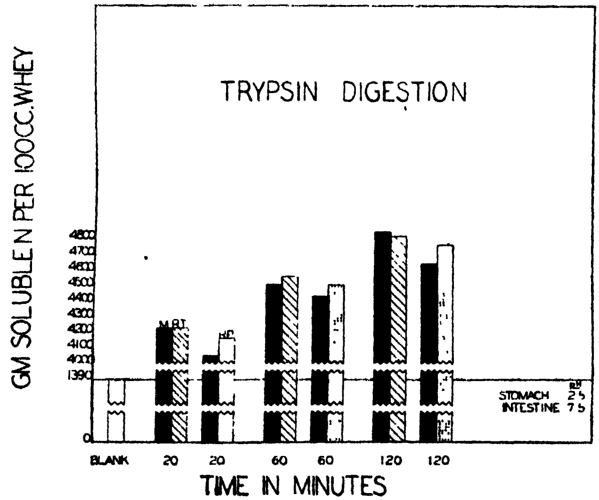

FIG. 1. Peptic-tryptic digestion of rennet-custard milks compared with pasteurized milks as controls. (Group 1 of the experiments, greatest amount of pepsin-hydrochloric acid and pancreatin-bile.) The pH in peptic digestion: 2.5; pH in tryptic digestion: 7.5; amount of pepsin used: 3 ml. 0.6% pepsin solution to 100 ml. milk; amount of trypsin (pancreatin-bile) used: 3 ml. of 3% solution of pancreatin-bile. (Average of 4-experiment series.) M = milk controls; RT = rennet-custard made with tablets; RP = rennet-custard made with powder.

serve for contrasting the breakdown rates of rennet-custards versus controls of untreated milk under a certain set of conditions, even though these conditions may not simulate exactly the conditions in the living body.

In the pepsin-hydrochloric acid digestion phase the rennet-custards produce curds which are smaller than those of the controls. These curd size differences may influence the digestion rates (4). In the later pancreatin-bile digestion, the coagula liquefy quickly and disappear. Only when the pepsin-hydrochloric acid and the pancreatin-bile preparations were both of low potency did curds of considerable size persist to the end of the process.

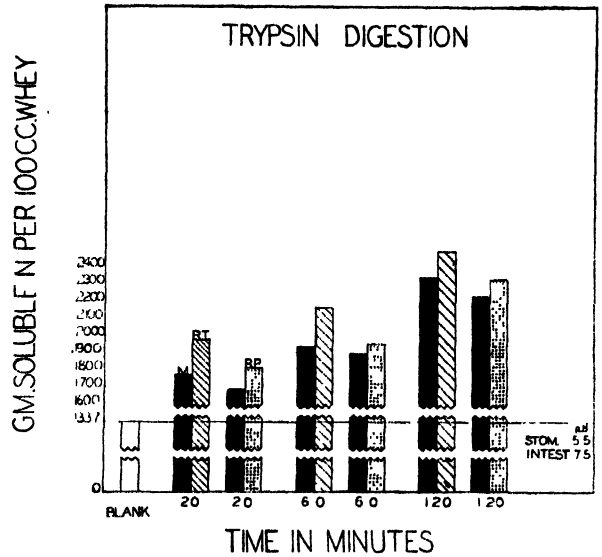

FIG. 2. Peptic-tryptic digestion of rennet-custards compared with pasteurized market milks as controls. (Group 3 of the experiments, smallest amount of pepsin-hydrochloric acid and pancreatin-bile.) The pH in peptic digestion: 5.5. Amount of pepsin ⅛, and of hydrochloric acid, ⅓ of amount used in group 1. Amount of (trypsin) pancreatin-bile: ¼ of amount used in group 1. (Average values from 6-experiment series.) M = milk controls; RT = rennet-custard made with tablets; RP = renent-custard made with powder.

Here, as with the pepsin-hydrochloric acid digestions, the curds from rennet-custards were smaller than those from the control milks.

With the "stronger" peptic-tryptic procedure, the difference between the rennet-custards and their controls was negligible (fig. 1, table 2).

With the "weaker" peptic-tryptic procedure, the rennet-custards as made with the two preparations of enzyme lead with a slight margin over the controls (fig. 2, table 3). The pepsin-hydrochloric acid phase of the digestion presented approximately the same relative picture, but of course with less total digestion, since the hydrogen ion concentration (pH 5.5) practically excluded any peptic activity.

With the "intermediate" peptic-tryptic procedure, the rennet-custards

TABLE 2

Pepsin-trypsin digestion in vitro

Group 1

Gastric digestion: 3 ml. pepsin solution (0.6%) pH: 2.5
Tryptic " : 3 ml. (trypsin) pancreatin-bile solution (3%) pH: 7.5

Average from 4-experiment series

Milk	Digestion time	Sol. N in whey from 100 ml. of milk	Increase of sol. N in whey from 100 ml. of milk	Difference	% Casein made sol.	Diff.
	min.					
Blank test	..	0.1390				
Control 1	20	0.4231	0.2841		73	
#1	20	0.4231	0.2841	0.0000	73	0
Control 3	20	0.4054	0.2664		68	
#3	20	0.4165	0.2775	0.0111	71	3
Control 1	60	0.4512	0.3122		80	
#1	60	0.4563	0.3173	0.0051	81	1
Control 3	60	0.4435	0.3045		79	
#3	60	0.4512	0.3122	0.0077	80	1
Control 1	120	0.4850	0.3460		89	
#1	120	0.4824	0.3434	0.0026	88	1
Control 3	120	0.4645	0.3255		84	
#3	120	0.4768	0.3378	0.0123	87	3
Genuine milk		0.5289			..	

TABLE 3

Pepsin trypsin digestion in vitro

Group 3

Gastric digestion: 0.5 ml. pepsin solution (0.6%) pH: 5.5
Tryptic " : 0.5 ml. (trypsin) pancreatin-bile solution (3%) pH: 7.5

Average from 6-experiment series

Milk	Digestion time	Sol. N in whey from 100 ml. of milk	Increase of sol. N in whey from 100 ml. of milk	Difference	% Casein made sol.	Diff.
	min.					
Blank test		0.1337		...		
Control 1	20	0.1771	0.0434		11	
#1	20	0.1979	0.0642	0.0208	17	6
Control 3	20	0.1685	0.0348		9	
#3	20	0.1815	0.0478	0.0130	13	4
Control 1	60	0.1936	0.0599		16	
#1	60	0.2160	0.0823	0.0224	22	6
Control 3	60	0.1887	0.0550		15	
#3	60	0.1946	0.0609	0.0059	16	1
Control 1	120	0.2338	0.1001		26	
#1	120	0.2483	0.1146	0.0145	30	4
Control 3	120	0.2226	0.0889		24	
#3	120	0.2324	0.0987	0.0098	26	2
Genuine milk	...	0.5126	...	...	...	...

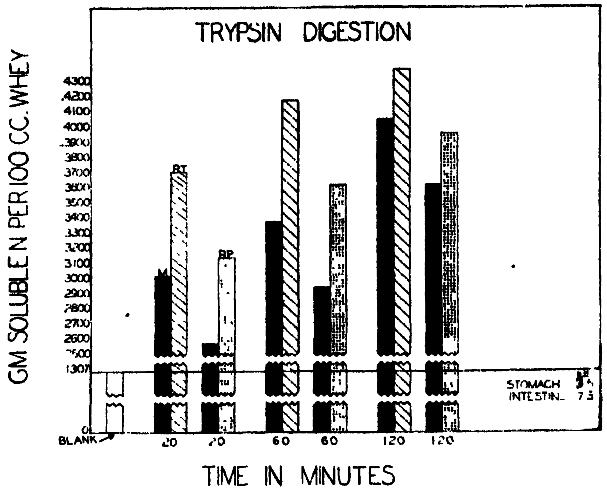

FIG. 3. Peptic-tryptic digestion of rennet-custard milks compared with pasteurized market milks as controls. (Group 2 of the experiments, intermediate conditions.) The pH in peptic digestion: 3.5. Amount of pepsin: ⅓, and of hydrochloric acid: ⅔ of amount used in group 1. Amount of pancreatin-bile (trypsin): ½ of amount used in group 1. (Average values from 30-experiment series.) M = milk controls; RT = rennet-custard made with tablets; RP = rennet-custard made with powder.

showed more casein digestion than the controls; this was appreciable at 20 and 60 minutes, but less striking at 120 minutes (fig. 3, table 4).

TABLE 4

Pepsin-trypsin digestion in vitro

Group 2

Gastric digestion: 1 ml. pepsin solution (0.6%) pH: 3.5
Tryptic " : 1.5 ml. (trypsin) pancreatin-bile solution (3%) pH: 7.5

Average from 30-experiment series

Milk	Digestion time	Sol. N in whey from 100 ml. of milk	Increase of sol. N in whey from 100 ml. of milk	Difference	% Casein made sol.	Diff.
	min.					
Blank test		0.1307		..		
Control 1	20	0.3030	0.1723		45	
#1	20	0.3717	0.2410	0.0687	63	18
Control 3	20	0.2580	0.1273		33	
#3	20	0.3145	0.1838	0.0565	48	15
Control 1	60	0.3382	0.2075		54	
#1	60	0.4188	0.2881	0.0806	76	22
Control 3	60	0.2954	0.1643	...	43	....
#3	60	0.3633	0.2326	0.0683	61	18
Control 1	120	0.4061	0.2754	.	72	...
#1	120	0.4398	0.3091	0.0337	81	9
Control 3	120	0.3633	0.2326		61	. .
#3	120	0.3971	0.2664	0.0338	70	9
Genuine milk	.	0.5117		. ..		. .

The greater relative degree of breakdown of rennet-custards in contrast to their controls of untreated milk in some of these experiments suggests that rennin facilitates in one way or another—perhaps by opening up some peptide bindings—the peptic-hydrochloric acid activity. The greatest differences between rennet-custards and controls did not appear when the peptic and tryptic activities were strongest, as in group #1 (although this group of course showed the largest total breakdown of casein for both), or when peptic and tryptic activities were weak, as in group #3, but when the peptic and tryptic activities were moderate, as in group #2. If it is permissible to draw a clinical parallel, then this enhanced rate of digestion of rennet-custards seemingly would take place under corresponding conditions in the human body also.

SUMMARY AND CONCLUSIONS

Rennet-custards and ordinary untreated milks were subjected to experimental study in vitro to investigate the possible influence of rennin upon digestibility. The degree of digestibility was measured by the amount of soluble nitrogen which appeared in the whey after the milks had been exposed to pepsin-hydrochloric acid (peptic) digestion followed by pancreatin-bile (tryptic) digestion.

Different combinations were investigated. When strong peptic digestion was followed by strong tryptic digestion, practically no differences between the two types of milk were detectable. The same result was obtained when both peptic and tryptic activity were markedly reduced. Under intermediate digestive conditions, however, faster digestion of the rennet-custards was noticeable. For example, after the first 60 minutes of tryptic digestion, an average of 18 per cent more casein was digested in the rennet-custards than in the controls. This difference became less marked after 2 hours of digestion.

It may be concluded, therefore, that under certain experimental conditions, rennin treatment of milk speeds up digestion. It seems likely that a similar effect would take place in vivo under corresponding conditions.

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BEST RECORDS VS. THE AVERAGE OF ALL RECORDS FOR THE EVALUATION OF A SIRE'S INHERITANCE FOR LEVEL OF PRODUCTION

R. R. GRAVES[1]

Most of the published data on which sires are proved is on the basis of averages of all records made by daughters and their dams. The sires proved in the dairy herd improvement associations, and probably in the herd tests of the breed associations, are on the basis of the averages of all the records made by the daughters and by their dams. Probably this use of the average of all records stems from the belief that a cow that has made several records has given better proof of her producing ability than the cow that has made only one record; that an average of a number of records is a better indication of the cow's real producing ability than one record; that some cows are primed for one big record and that such a record is far above her ability to produce year in and year out. The publicity that has been given to the cows making big lifetime records has probably added to the belief that the average of all records is the best medium to use for evaluating the inheritance of a sire. Most of the proof for sires is on young sires and consequently the averages usually represent a far greater number of records for the dams than for the daughters. Not infrequently the daughters may average only one record apiece while the average number for the dams may be for from 1 to 6 or more records.

In studying the proof on a large number of sires being considered for use in the Bureau of Dairy Industry's experimental breeding herds, we have been impressed with the number of sires that show up very favorably when their proof is based on the averages of several records for the dams and only one or two records for the daughters, and that show up very poorly when the proof is based on the best record made by the daughter and the best record made by the dam.

This is illustrated by the proof obtained on 16 sires loaned to cooperators by the Huntley Experiment Station. The data for these 16 sires are shown in table 1 and so arranged that a comparison is available of the average butterfat yields of the daughters, and their dams, for each sire, on the basis of both the averages of their best records and the averages of all their records. The number of daughter-dam pairs for each sire; the number of daughters that made records that were better than those of their dams, in the comparison of the best records; the number of records made by the

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TABLE 1

Comparison of the best records and the average of all records of the daughter-dam pairs of 16 bulls from the Huntley Experiment Station

Sire number	Best records				All records average			
	Number of pairs	Drs. ave. fat.	Dams ave. fat.	Number better	Number of records	Drs. ave. fat	Dams ave. fat	Number better
191	29	654	620	18	49–52	631	596	17
193	16	416	357	13	24–18	395	355	11
194	5	413	459	2	5–18	413	406	3
195	14	744	675	8	19–26	734	650	11
199	6	407	416	4	6–16	407	377	4
505	10	350	398	1	10–32	350	353	4
503	16	487	451	8	25–63	465	422	8
506	13	446	507	4	24–66	424	418	7
508	11	511	530	4	18–50	484	450	7
510	7	404	408	5	7–8	404	406	5
517	6	421	432	3	8–17	415	392	4
520	7	386	384	5	7–25	386	324	5
521	6	376	454	0	6–20	376	385	3
523	5	288	347	1	5–20	288	286	2
528	7	717	579	5	8–8	704	578	5
531	6	452	470	3	6–32	452	419	4

daughters and by the dams in the comparison of the averages of all records; and the number of daughters whose average of all their records was greater than the average for all the dams' records, is also shown in table 1. For instance, sire 191 has 29 daughter-dam pairs but in the comparison of averages of all records the daughters have 49 records and the dams have 52 records, the number of records by daughters and dams being approximately equal. Sire 503 has 16 daughter-dam pairs, and in the comparison based

TABLE 2

The per cent increases and the sire indexes, calculated from the best record, and also from the average of all records, as given in table 1

Sire	Per cent increase on best records	Bull index best records	Per cent increase all records	Bull index on all records
191	+ 3.5	684	+ 5.9	666
193	+16.5	475	+11.2	435
194	−10.0	367	+ 1.7	420
195	+10.2	813	+12.9	818
199	− 2.1	398	+ 7.9	437
505	−12.1	302	− 0.8	347
503	+ 8.0	523	+10.2	508
506	−12.0	385	+ 1.4	430
508	− 3.6	492	+ 7.6	518
510	− 0.9	400	− 0.5	402
517	− 2.4	410	+ 5.9	438
520	+ 0.5	388	+19.1	448
521	−17.2	298	− 2.2	367
523	−17.0	229	+ 0.7	290
528	+23.8	855	+21.8	830
531	− 3.8	434	+ 3.8	485
Average 16	− 1.22	465.7	+ 6.66	490.0

on averages of all records the daughters have a little less than two records per daughter, while the dams have almost four records per dam. In the case of sire 506 the dams average five records per dam. In the comparison for sire 531 the daughters average one record apiece and the dams average a little better than five records per dam.

The first 11 sires in table 1 are all sons of one sire; the last five are all sons of another sire.

In table 2, the data in table 1 are used to show the per cent increase in butterfat yield when the best records of daughters and dams are compared, and an equal parent sire index is calculated from the same data. The per cent increase in butterfat yield and an equal parent sire index is also shown for the data based on the average of all records made by daughters and dams. In general the data in table 2 indicate that where the number of records of the dams greatly exceeds the number of records of the daughters, in the comparison based on averages of all records, the per cent increase of the daughters over the dams and also the sire index will be much greater than the per cent increase, and the sire index, for these same animals when the comparison is based on the best records of the daughters and the dams. Note the occurrence of these increases in sires 194, 199, 505, 506, 508, 517, 520, 521, 523 and 531. There are two exceptions, namely, sire 195, where the dams have 26 records and the daughters 19 and the per cent increase is only 2.7 and the sire index is raised only 5 pounds; and 503, where the 16 dams have 63 records and the 16 daughters 25 records, and the per cent increase is 2.2 and the sire index is decreased 15 pounds. The case of 503 is most unusual

The reverse of this trend would be expected where the daughters have a greater number of records than the dams. Only one sire, 193, illustrates this. He has 16 daughters with 24 records and the 16 dams have only 18 records, and table 2 shows that the per cent increase and the sire index based on the average of all records is smaller than the per cent increase and the sire index that is based on the comparison of the best records of the daughters and the dams.

If there is no great difference in the number of records of the daughters and dams, and if all the records are made under the same conditions there will be no great difference in the per cent increase and the sire index secured by the two methods of comparison.

This trend is probably the result of the fact that where a cow is tested year after year she is almost certain to have some bad years when her production will be below her best, and these poor years will reduce her average yield. These poor years are not necessarily due to a poor inheritance. They may be due to her having been bred too soon after calving; to an attack of mastitis; to bloat or poor hay or failure to clean properly after calving, or any one of a dozen different things that may result in her failure to produce up to her inherent capacity. On the other hand the two-year-old daughters are more likely to be sound in their first lactation periods than at any other time in their lives, and barring lack of development or the possession of an inheritance for slow maturity, they are likely to produce at as high a level in their first lactation period—age considered—as in any subsequent lactation. Therefore, if the proof of a bull is based on the average production of his daughters in their first lactation periods as compared to an average for all the records made by their dams, that comparison is likely to be unduly favorable to the sire. It is a much more stringent test for the sire to base his proof on the best records made by his daughters as compared to the best records made by their dams.

SUMMARY AND CONCLUSIONS

Daughter-dam comparisons for 16 sires are made, one comparison on the basis of the best records made by the daughters and the best records made by the dams, and a second comparison on the basis of the average for all records made by the daughters and all records made by their dams.

The per cent increase in butterfat yield and an equal parent sire index is shown for each sire and for each of the two comparisons.

When the number of records averaged for the dams is larger than the number of records averaged for the daughters the comparison based on averages is more likely to be unduly favorable to the sire than is the comparison based on the best record made by the daughters and the dams. This is due to the fact that any cow that has a number of records is likely to have some lactations when her production is below her best level and these low years reduce her average yield. The comparison is likely to be particularly favorable to the sire if his daughters' first records, made under favorable conditions, are compared to an average of several records made by the dams.

The comparison of the best records of the daughters and the best records of the dams is a much more rigorous test for the evaluation of the sire's inheritance for level of production. There will be fewer disappointments where sires are being selected for the improvement of germ plasm for high levels of production if they are selected on the basis of the best records rather than on the basis of the average of all records.

THE RELATION OF INCLINATION OF RUMP TO INCLINATION OF UDDER, PRODUCTION ABILITY AND BREEDING EFFICIENCY[1]

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In many cows the pin bones are lower than the hip bones, resulting in what is commonly called a "sloping rump". Perhaps the frequency of the sloping rump is decreasing somewhat. Certainly most breeders have discriminated against the sloping rump in their selection of breeding animals. Man's idea of beauty in the bovine long ago insisted that the rump should be level, in spite of the fact that most species of animals possess sloping rumps. Note the rarity of the level rump in the animals in the zoo or in the wild. Probably because the level rump was associated in man's mind with beauty, there have been developed over a period of years opinions that there are certain associated defects that are likely to accompany the undesirable sloping rump, some of them of economic importance. One such defect that received emphasis was that if the rump slopes the floor of the udder will be tilted because the floor of the udder parallels the rump, with a likelihood of the fore quarters being undeveloped. The following quotation from Van Pelt's Cow Demonstration emphasizes this idea.

> Many cows, though long in the rump, droop from the hip bones to the pin bones and are described by the expression "Drooping rumped." This conformation not only detracts from the beauty of the cow but as a rule those cows which droop at the rump, also have tilted or slanting udders a portion of which seems to have been cut away and this naturally detracts from the ability of the cow.
>
> On the other hand those cows which carry out straight from the hip bones to the pin bones have udders that are straight on the bottom, symmetrical and carry well forward with each quarter large and uniform in size. The fact that the length of udder can be determined by the length from hip bones to the pin bone, and the shape of the udder by the manner in which the rump is carried out, is likely due to the law of correlation of parts which enables the anatomist when he finds a bone to determine from its dimensions the dimensions of every other bone in the animal's body from which it came.

Few, if any, of these opinions have been checked by recorded observations and statistical analysis. There existed in the files of the United States

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[1] The original data used in this paper were in a thesis submitted to the Graduate School of the Oklahoma Agricultural and Mechanical College in partial fulfillment for the degree of Master of Science by R. E. Leighton. This paper presents a new analysis of the original data and some new material.

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Dairy Experiment Stations, where breeding experiments have been carried on over a period of years, carefully made photographic records of the types of the animals that have constituted several generations of planned breeding that could be analyzed for the purpose of determining the relation of the angle of the rump to other body characteristics and functions. The material for this study has been confined to the records that were available at the Woodward, Oklahoma, Station.

PROCEDURE

A series of more than 500 photographs of 155 Holstein-Friesian females in the herd of the U. S. Dairy Experiment Station at Woodward were made in connection with the inheritance investigations in progress at that station over a period of some 15 years. All females except those that die or become non-breeders are kept in the herd until they have completed at least one 365-day lactation record on official test. The size of the herd that can be maintained at the station is limited by both facilities for handling and funds. These factors have made it necessary to dispose of many of the animals by the time they are 5 years of age. Consequently the animals studied in this project were largely young cows, and they constitute an unselected cow population.

Each cow was photographed each year by a method that made all photographs comparable in scale and detail. The animals were posed on a level track, squarely in front of the camera which was mounted on a fixed post at a distance of 28 feet. No attempt was made to pose the animals so as to cover up defects; but a standardized position was used that was designed to show the actual conformation to the greatest possible extent. The same experienced photographer made and developed all of the pictures and the same camera and lens were used for the entire period. The prints were 5 by 7 inches in size and clear in detail. Legends were placed on the pictures when made. As the photographs were all made in the fall of the year it was not possible to get pictures of all of the animals when at the same stage of lactation.

In order to measure the angle of inclination or slope of the rump and udder a base line was established by drawing a line through the extreme upper attachment of the dew-claws on the front and rear legs nearest the camera. Since the cows were standing directly in front of the camera and on a level track, this proved a satisfactory line from which to measure the angles. To obtain the angle for the rump slope, a line was drawn through points at the dorsal extremity of the hip bone (tuber coxae) and the dorsal extremity of the pinbone (tuber ischii). The angle formed by the intersection of this line with the base line, or a line parallel to the base line, was measured with a protractor. To measure the udder slope, a line was drawn through the points where the front portion of the front teat and the front portion of the rear teat join the udder. The line was extended until it intersected the base line and the angle formed was measured with a protractor. If it was evident that the pose of an animal was unnatural the measurements for that animal were discarded. If the udder was not lactating or if it appeared to be abnormal, the udder measurements were not used. The photographs were measured during the winter of 1940 by R. E. Leighton and, as a check, a number were measured again a year later. In all cases the rump slope readings were the same, but in a few cases there was as much as one degree variation in the udder slope readings.

The last animal photographs were made in 1938. In 1941 and 1942 measurements were made with a clinometer of the angle of the rump of 17 living animals in the herd. The slope of the rump for these same animals had been calculated from their pictures, though the pictures were made at an earlier age. The measurements on the living animal offer a check on the accuracy of the slopes calculated from the photographs. The results are shown in table 1, the readings made on the living animals appearing in parentheses. In view of the difference in age of the animals when photographs were taken and when the readings on the living animals were made, the results arrived at by the two methods appear quite consistent.

TABLE 1

A comparison of the degree of inclination of rump as taken from photographs and as secured from living animals

Cow number	Age of animal—years							
	1	2	3	4	5	6	7	8
	Degree of inclination							
W-212	7	11	12	15	10		(10)	(11)
W-223	12	11	12	12			(12)	(11)
W-235	11	11	13			(13)	(14)	
W-245	9	11	14		(13)	(12)		
W-246	9	10			(10)	(10)		
W-247	11	10			(9)	(8)		
W-251	10	7		(8)				
W-256	10			(11)	(10)			
W-258	13		(13)	(11)				
W-259	10		(7)	(12)				
W-260	12		(11)	(13)				
W-261	10		(12)	(10)				
W-262	15		(15)	(16)				
W-263	12		(12)	(10)				
W-264	8		(11)	(12)				
W-265	10		(12)	(12)				
W-266	9		(9)	(13)				

The figures enclosed in parentheses are those taken on live animals in February, 1941 and 1942.

Only two cows had both picture measurements and clinometer readings of udder slope and these were taken 3 years apart. The photographs taken when the two cows were 3 years old showed 11° and 32° slope, respectively.

The clinometer readings made 3 years later were 23° and 32°, respectively. The increase in udder slope of the first cow was probably due to the effect of advancing age and does not indicate a lack of consistency in the slope calculated from the picture and the direct reading on the living animal.

RESULTS AND DISCUSSION

Effect of age. To determine the effect of age on the slope of rump and the slope of the udder, averages were computed for the readings for all animals for each year, starting at 1 to 6 months for slope of rump, and at 19 to 30 months for slope of udder. Since the animals were photographed each year as long as they were in the herd, many of the animals are represented in each age-year group, but the younger groups are represented by larger numbers. In order to obtain data that would bear more specifically on the effect of advance in age on the slope of rump and of udder, an additional calculation was made on 20 cows on which there were data for each age year up to 5 years, with the exception that some of these animals were not represented by pictures for study in the youngest age groups. These two groups of data are shown in table 2, A and B.

TABLE 2

Effect of age upon inclination of rump and udder

Age in months	Rump		Udder	
	Animals	Inclination	Animals	Inclination
A—Average of all animals				
	number	*degrees*	*number*	*degrees*
1–6	76	10.8		
7–18	124	11.4		
19–30	115	12.2	50	10.1
31–42	98	13.2	82	14.1
43–54	64	13.1	59	14.7
55–66	36	14.1	33	18.3
67–78	20	13.4	18	18.0
79–90	12	13.4	12	19.0
B—Average of 20 animals with complete data from 1 to 5 years of age				
1–6	8	9.4		
7–18	20	10.7		..
19–30	20	12.0	12	9.7
31–42	20	12.7	20	14.2
43–54	20	13.0	20	16.3
55–66	20	13.3	20	18.3

In general the two groups show the same trend but there is some difference in magnitude of the increase in degree of slope of the rump with age. In the group shown in table 2, A, the maximum slope in the rump is attained in the age group 55–66 months, with a per cent increase in the degree of slope from the age group 1–6 months, of 30.6. The maximum degree slope for the 20-cow group is also reached at 55–66 months and the total per cent increase from the 1–6 months is 41.5. There is a fairly consistent increase in the slope of the rump with each age group, with the most rapid increases in the A group occurring between 7–18 months and 19–30 months, with a 7 per cent increase; and from 19–30 months to 31–42 months, with an 8.2 per cent increase. In the B group the most rapid rate of increase came earlier. From 1–6 to 7–18 months the increase in degree of slope was 13.8 per cent; from 7–18 to 19–30 months, it was 12.1 per cent; and from 19–30 to 31–42 it was 5.8 per cent. Thereafter the per cent increase in slope of rump for each age group was 2.3 per cent.

The increase in degree of udder slope was much more pronounced. For the A group the maximum degree of slope was at 79–90 months and the total increase in degree of slope at this age from the slope at 19–30 months was 88.1 per cent. For the B group the maximum degree of slope for the 20 cows was reached at 55–66 months and the total increase in degree of slope at this age was 88.6 per cent over that at 19–30 months. In both groups the greatest increase in degree of slope came from 19–30 to 31–42 months with 39.6 and 46.4 per cent, respectively. For succeeding age groups the rate of increase was very irregular, being 4.2, 24.5, –1.6, and 5.5 per cent for the animals in table 2, A; and 14.8 and 24.5 per cent for those in table 2, B.

Thus it appears that in this herd there was an increase in slope of rump up to 5 years of age, with the most rapid increases in slope falling between 1 and 3 years of age. There was an increase in slope of udder up to 7 years, with the most rapid increases taking place between 2 and 3 years of age. The maximum degree of slope of the udder attained at mature age was approximately 40 per cent greater than the maximum degree of slope of the rump at mature age.

The above observations have applied to the averages secured from all animals in each age group. There were many individual animals that varied from this trend. It was observed that in some cases there appeared to be an improvement in the levelness of the rump, even when the measurements showed an actual increase in the degree of slope. The apparent improvement was probably due to a raised tail setting.

The relation of rump inclination to udder inclination. It has been pointed out that there is a common belief that the sloping rump is associated with the tilted udder. To determine whether there was any relationship between sloping rumps and tilted udders in the animals in the Woodward Station herd, correlation coefficients were run on 87 pairs, using the readings for animals in the 31–42 months age-group. This group has the largest population for udder inclination and the third largest population for rump inclination (see table 2, A). The coefficient secured was +0.021 ± 0.11, indicating that there is no relationship between the sloping rump in the animals in this study and the tilted udder. Correlation coefficients were run on these same 87 pairs to determine if there was any relation between the sloping rump and producing ability; or between the tilted udder and producing ability. The coefficient for sloping rump and producing ability was − 0.013 ± 0.107, indicating that the relationship was not significant. The coefficient for tilted udder and producing ability was − 0.196 ± 0.103, indicating that this relationship was not significant.

Thus it appears that in this group one is as likely to find a tilted udder on a cow with a level rump as on a cow with a sloping rump; or a cow with a sloping rump is no more likely to possess a tilted udder than is the cow with a level rump. Furthermore, producing ability, at least within the levels of production possessed by the cows in this study, is not effected by the degree of slope of either the rump or the udder.

Some observers believe that cows that have sloping rumps are more likely to have difficulty delivering calves than are cows with level rumps. Our records were not complete enough to permit a study of the relationship of degree of slope of rump to difficult calving, but we did run a correlation coefficient on the relationship of degree of slope of the rump to breeding efficiency, as measured by the number of services per conception. It is recognized that there are many factors that may cause a lowered breeding efficiency and it was hardly expected that the correlation coefficient for sloping rump and breeding efficiency would be significant. The coefficient was + 0.220 ± 0.088. While not high enough to be considered significant, it indicated a more pronounced trend than the other three coefficients that have been discussed.

The inheritance of the degree of rump and udder inclination. It appears from the data presented that neither the sloping rump nor the tilted udder has any influence on the producing ability of an animal. Also the levelness of the rump, or the degree of its slope, is not associated with either the levelness or the degree of tilt of the udder. Nevertheless animals with level rumps and/or level udders have much greater sales value than do animals that have sloping rumps and/or tilted udders, other points being equal. This will be true even though it is fully understood that these features do not have a bearing on producing ability, because of the fact that the level rump and the level udder are commonly associated with the correct type or beauty of an animal.

So far as is known there are no environmental conditions that will cause a level rump to become sloping. Photographic records in the Bureau of Dairy Industry, portraying the development of experimental breeding animals from calfhood to maturity, do show, however, that many animals undergo startling changes in the conformation of the rump. Some that are level become sloping and some that are sloping become level. The reasons for these changes are not known.

There are a number of known causes for changes in the shape of the udder. Injury or infection in one or more quarters may cause atrophy of the tissues that may result in an unbalanced udder. Investigations have shown that the average cow secretes in the rear quarters approximately 60 per cent of the total amount of milk produced. The greater weight of the milk in the rear quarters may result in a more pronounced sagging in these quarters. Then there is the not inconsiderable weight of the udder that may cause a relaxation of the supporting tissues. The average weight of 17 empty udders from cows at the Beltsville station that had been in milk 3 months or less at the time of slaughter was 72.98 pounds.

An investigation of the "Arrangement of the tissues by which the cow's udder is suspended,"[4] by W. W. Swett, P. C. Underwood, C. A. Matthews, and R. R. Graves, showed that probably the main supporting tissue of the udder was the fan-shaped septum between the two halves of the udder that attaches directly to the abdominal wall. If this main supporting tissue, or the lesser supporting tissues, becomes lax and stretches, because of hereditary tendencies, old age, or ill health, the shape and the balance of the udder may change quickly. The illustrations of these tissues in the publication cited show more clearly than can words how this may occur.

There was a greater probability of error in measuring the inclination of the udder than in measuring the inclination of the rump. This was because of the short distance between the base points—the base of the front and rear teats—from which the angle of the inclination of the udder was projected; the difficulty in accurately locating the teat base with some types of teats, especially those that are inclined to balloon or funnel out; and the differences that existed in the stage of lactation of different animals when photographed. The greater possibility of environmental factors influencing the shape of the udder and the greater probability of error in obtaining the correct angle of the inclination of the udder result in greater variations and more indefinite trends in the following study of the inheritance of the udder inclination than is the case in the study of rump inclination.

The data obtained in this study have been analyzed for indications of the part that inheritance plays in determining the extent of the inclination of the rump and the udder.

Table 3 shows the average inclination of the rump and of the udder of the daughters and of their dams, of the five sires used in the station herd. The data for the outbred and inbred daughters are shown separately. The program of taking photographs of each animal in the breeding herd each year had to be discontinued a few years ago, so that data are not available for all the daughter-dam pairs for sires 4 and 5. In reading the per cent increase or decrease in inclination in this table it should be kept in mind that a plus per cent indicates that the degree of slope of the daughters has increased over that of their dams, and the minus per cent indicates the degree of slope of the daughters has decreased or the rump or udder of the daughters has become more level as compared to that of the dams.

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[4] Journal of Agricultural Research, Vol. 65, No. 1.

The inbred daughters of sire 1 and the outbred daughters of sire 2 had the most sloping rumped daughters, each with an average of 13.5°; but the degree of slope of sire 1's inbred daughters was 10.6 per cent greater than the slope of their dams, while the degree of slope of sire 2's daughters was only 3.9 per cent greater than that of their dams. Sire 5's daughters had the least slope of any sire's daughters and they had 27.6 per cent less slope than did their dams. This was the greatest change from the average inclination of the rump of the dams shown by any group of daughters. Sires 3 and 4 were mated to dams that had the highest average rump inclination and sire 3's outbred daughters average 6.6 per cent less inclination than their dams and sire 4's daughters averaged 12.6 per cent less than their dams.

The per cent changes in udder inclination were much greater than those for the rump. Perhaps this is because the changes in udder inclination were due to some extent to environmental factors.

The greatest increase in udder inclination was in the daughters of sire 1. The dams of his outbred daughters had more level udders than did any other group of dams. The inbred daughters of sire 1 had udders with a greater inclination than did his outbred daughters, but the per cent increase in inclination was less.

Coefficients of correlation indicated that there was no relationship between the sloping rump and the tilted udder. The results shown in table 3 show why a correlation coefficient for these characters would be without significance. Sire 2's outbred daughters ranked with sire 1's inbred daughters in possessing the most sloping rumps but sire 2's daughters also had the most level udders. He increased the degree of inclination of rump of his daughters over that of their dams by 3.9 per cent and he decreased the udder inclination of his daughters 23.8 per cent. On the other hand, sires 3 and 4 decreased the rump inclination of their daughters and greatly increased their udder inclination.

From the data in table 3 equal parent indexes have been calculated for each sire for inclination of rump and for inclination of udder, on the assumption that the average inclination of the rump or udder of the daughters represents the average of the degree for that character transmitted by the parents. These indexes for sires 1, 2, and 3 are used in table 4, along with the measurements of certain cows that they were mated to, to show the expected inheritance for inclination of rump and udder as compared to that actually secured in full sisters. Thus the index for inclination of rump calculated from the outbred daughters of sire 1 and their dams is 12.8°, and the index for inclination of udder is 27.2°. Sire 1 was mated to cow W–14, whose inclination of rump was 9.0°, and of udder 1.0°. The average of sire

TABLE 3

Comparison of degree of rump and of udder inclinations of daughters–dams of five sires

Sires	Number pairs	Rump inclination			Udder inclination		
		Daughters	Dams	Increase or decrease of daughters	Daughters	Dams	Increase or decrease
		degrees	*degrees*	*per cent*	*degrees*	*degrees*	*per cent*
No. 1, outbred	5	11.8	10.8	+ 9.2	17.6	8.0	+120.0
No. 1, inbred 75%*	9	13.5	12.2	+10.6	22.0	14.3	+ 53.8
No. 1, inbred 87.5%†	1	16.0	17.0	− 5.9	20.0	22.0	− 9.0
No. 2, outbred	20	13.5	13.0	− 3.9	9.3	12.2	− 23.8
No. 2, inbred 62.5%‡	1	12.0	10.5	+14.3	12.0	20.0	− 40.0
No. 3, outbred	18	12.7	13.6	− 6.6	16.1	11.0	+ 46.3
No. 3, inbred 75%	3	13.0	14.6	−10.9	13.3	14.0	− 5.0
No. 4, outbred	4	11.8	13.5	−12.6	19.7	13.0	+ 51.5
No. 5, outbred	8	7.6	10.5	−27.6	11.0	§	

* 75% inbreds, result of mating daughter to sire.

† 87.5% inbreds, result of mating 75% inbred daughter to sire—3 crosses of sire.

‡ 62.5% inbreds, result of mating granddaughter to sire.

§ Pictures on dams of daughters as 3-year-olds not available for measuring inclination of udder.

TABLE 4

Comparison of degree of rump and of udder inclinations of full sisters with parent index

Sire and dam	Rump inclination (degrees)		Udder inclination (degrees)	
	Parents	Daughters	Parents	Daughters
Sire No. 1	12.8		27.2	
Dam W-14	9.0		1.0	
Equal parent index	10.9	W-36, 14; W-41, 8	14.1	W-36, 10; W-41, 19
Sire No. 2	14.0		6.4	
Dam W-14	9.0		1.0	
Equal parent index	11.5	W-77, 13; W-63, 12; W-88, 8	3.7	W-77, 8; W-63, 18; W-88, 11
Sire No. 1	12.8		27.2	
Dam W-16	10.0		10.0	
Equal parent index	11.4	W-39, 12; W-47, 8	18.6	W-39, 23; W-47, 25
Sire No. 2	14.0		6.4	
Dam W-18	14.0		11.0	
Equal parent index	14.0	W-68, 11; W-81, 17	8.7	W-68, 0; W-81, 12
Sire No. 2	14.0		6.4	
Dam W-23	9.0		13.0	
Equal parent index	11.5	W-69, 13; W-79, 11	9.7	W-69, 8; W-79, 0
Sire No. 1 (inbred)	14.8		29.7	
Dam W-25	15.0		11.0	
Equal parent index	14.9	W-42, 16; W-33, 10	20.3	W-42, 22; W-33, 14
Sire No. 2	14.0		6.4	
Dam W-25	15.0		11.0	
Equal parent index	14.5	W-80, 13; W-86, 11; W-98, 16	8.7	W-80, 12; W-86, 2; W-98, 15
Sire No. 2	14.0		6.0	
Dam W-38	17.0		11.0	
Equal parent index	15.5	W-93, 13; W-54, 17	8.7	W-93, 13; W-54, 22
Sire No. 2	14.0		6.4	
Dam W-44	18.0		18.0	
Equal parent index	16.0	W-94, 14; W-223, 12	12.2	W-94, 2; W-223, 0
Sire No. 3	11.8		21.0	
Dam W-55	12.0		9.0	
Equal parent index	11.9	W-226, 9; W-95, 14	15.0	W-226, 17; W-95, 12
Sire No. 3	11.8		21.0	
Dam W-77	13.0		8.0	
Equal parent index	12.4	W-213, 14; W-99, 14; W-233, 10	14.5	W-213, 19; W-99, 12; W-233, 19
Sire No. 3	11.8		21.0	
Dam W-79	11.0		0.0	
Equal parent index	11.4	W-219, 10; W-203, 11	10.5	W-219, 10; W-203, 14

1's index for inclination of rump, and of cow W–14's actual inclination of rump, was 10.9°, the average inheritance for inclination of rump from these two parents. Inclination of the rump of the two full sisters resulting from the mating of these two parents, W–36 and W–41, is 14° and 8°, respectively. Likewise, the average index for these two parents for inclination of udder is 14.1 and the inclination for udder of W–36 and W–41 is 10° and 19°, respectively.

Such comparisons can only be of value in showing trends for the reasons that both parents were undoubtedly heterozygous for the factors determining degree of slope and consequently no one index figure can represent the range that they will transmit at different matings; and also because the index as calculated for the sire is a genotypic expression of his inheritance for these characters, while the measurement of the dam and of each daughter for degree of inclination is a phenotypic expression. Nevertheless the data in this table do show trends that appear to indicate that the inclination of the rump, and to a less extent the udder, is controlled largely by inheritance.

TABLE 5

The "expected" inheritance from the parents and the "actual" phenotypic inheritance received, as shown by the average inclinations of full sisters

Parents	Inclination of rump		Inclination of the udder	
	Expected index	Actual average of daughters	Expected index	Actual average of daughters
	degrees	*degrees*	*degrees*	*degrees*
1 × 14	10.9	11.0	14.1	14.5
2 × 14	11.5	11.0	3.7	12.4
1 × 16	11.4	10.0	18.6	24.0
2 × 18	14.0	14.0	8.7	6.0
2 × 23	11.5	12.0	9.7	4.0
1 × 25	14.9	13.0	20.3	18.0
2 × 25	14.5	13.3	8.7	9.7
2 × 38	15.5	15.0	8.7	17.5
2 × 44	16.0	13.0	12.2	1.0
3 × 55	11.9	11.5	15.0	14.5
3 × 77	12.4	12.7	14.5	16.7
3 × 79	11.4	10.5	10.5	12.0
Average	12.9	12.2	12.0	12.5

The data in table 4 give some idea of the variability of full sisters for these two variables. The daughters of W–14 and W–25 are especially interesting because each of these dams has daughters by sires 1 and 2. The daughters of cow W–25 by sire 1 are inbred and the index for sire 1 in this case is calculated from the averages of his 9 inbred daughters and their dams. Note the variability in the udder inclination of the three daughters of sire 2 and W–14, though the index of the parents was only 3.7. Also note the low range of variability of udder inclination in the two daughters of sire 2 and cow W–44, though the parent index was 12.2.

In order to show the trend more clearly the data in table 4 are condensed and given in table 5 as the "expected" and the "actual" inclinations occurring in the daughters.

The expected inheritance for inclination of rump for the 12 parental combinations shown in table 5 varies from 10.9 to 16.0°. As is to be expected in the matings of heterozygous parents, there is considerable variation among the full sisters resulting from each parental combination. When, however, the readings for the full sisters are averaged, the results fit quite closely to the expected inheritance for inclination of rump. Thus the "expected" for the first 2 parents is 10.9° and the average of their 2 daughters is 11.0°; and for each of the other parental combinations the results are: 11.5 to 11.0, 11.4 to 10.0, 14.0 to 14.0, 11.5 to 12.0, 14.9 to 13.0, 14.5 to 13.3, 15.5 to 15.0, 16.0 to 13.0, 19.9 to 11.5, 12.4 to 12.7, and 11.4 to 10.5°. The average for the expected for the 12 parental combinations is 12.9° and the average of the full sisters of the different parental combinations is 12.2°. Thus it appears that inclination of rump is clearly an inherited character; and of a multiple factor type.

The "expected" inheritance for inclination of udder covers a much wider range than the "expected" inheritance for inclination of rump, ranging from 3.7 to 20.3°. There is also a greater departure from the "expected" in both the individual daughter and in the average of the full sisters. This is to be expected in view of the greater part that environment may play, as well as the possibility of greater error in measurement. Though there is not as close a fit of the "actual" to the "expected" as in rump inclination, the trends of the "actual" and "expected" are in sufficient agreement to indicate that inheritance plays a major part in determining the extent of the inclination of the udder.

The comparison of the "expected" to the "actual" as expressed by the average of the degree of udder inclination of the full sisters from each parental combination, reading from top to bottom, is as follows (table 5): 14.1 to 14.5, 3.7 to 12.4, 18.6 to 24.0, 8.7 to 6.0, 9.7 to 4.0, 20.3 to 18.0, 8.7 to 9.7, 8.7 to 17.5, 12.2 to 1.0, 15.0 to 14.5, 14.5 to 16.7, and 10.5 to 12.0°. The average for the "expected" is 12.0° and for the actual 12.5°. The daughters of sire 2 and W–14 greatly exceed the parental index; also the daughters of sire 2 and W–38. The 2 daughters of sire 2 and W–44 and the daughters of sire 2 and W–23 have much more level udders than was to be expected from the parental index. On the whole, however, where the parental index is high the average of the daughters is high, and where the parental index is low the average of the daughters is low. It appears that the degree of slope of the udder is also a blended inheritance but that the inheritance picture is far more likely to be distorted by environmental factors than is the degree of slope of the rump.

TABLE 6

Variation in rump and udder slope and milk and butterfat production by groups according to sire of the groups

Group	Average rump slope, age 3 years			Average udder slope, age 3 years			Average production, mature equivalent				
	Animals	Degrees	Coefficient of variation	Animals	Degrees	Coefficient of variation	Animals	Milk	Coefficient of variation	Butter fat	Coefficient of variation
	number						*number*	*pounds*		*pounds*	
Daughters of sire 1	14	11.3	28.3	14	12.8	50.0	14	18,735	11.9	595	12.0
75 per cent inbred daughters of sire 1	9	13.6	19.2	9	22.0	25.4	9	16,321	22.0	509	23.0
Daughters of sire 2	20	13.9	25.2	20	11.0	55.0	20	20,819	12.7	685	11.0
Daughters of sire 3	22	12.8	18.7	22	15.0	36.0	22	17,458	20.2	624	19.9
75 per cent inbred daughters of sire 3	4	12.0	6.7	4	14.0	18.0	3	16,418	5.3	607	12.2
Daughters of sire 4	6	13.0	25.3	6	18.0	45.0	6	18,896	9.4	678	12.1
Daughters of sire 5	6	8.6	39.5	6	11.0	17.3	3	17,718	7.2	706	6.6

Most of the records were made between 2 and 3 years of age.

VARIABILITY

Coefficients of variability were calculated for the daughters of the five sires, on which data were secured on the inclination of the rump and the udder, for milk yield, butterfat yield, inclination of the rump, and inclination of the floor of the udder. Coefficients for these characters were calculated separately for the inbred daughters of sires 1 and 3. These coefficients are shown in table 6 along with average inclination of the rump and of the floor of the udder, and the average milk yields and the average butterfat yields of the daughters of the five sires.

The outbred daughters of sire 1 were less sloping on the average than his inbred daughters, but the variation in degree of slope was far greater in the outbred daughters. His inbred daughters also had less variability in slope of the udder floor. In this case inbreeding brought about a more uniformly sloping rump and a more uniformly sloping udder floor, but when it came to milk yield and butterfat yield the inbred daughters had lower yields, and the variability of the yields was far greater than that of the outbred daughters. The greater variability in milk and butterfat yields in the inbred daughters is in accordance with findings in an experiment with inbred dairy cattle at the Beltsville Station of the Bureau of Dairy Industry, the results of which will be published in a technical bulletin of the United States Department of Agriculture. On the other hand, the variability was lower in the inbred daughters of sire 3 than in his outbred daughters for all four variables. However, there were milk and butterfat yields on only three daughters.

The daughters of sire 5 had the lowest average slope of rump of the daughters of the five sires, but the coefficient of variation was the greatest. The daughters of sire 5 and the daughters of sire 2 had the lowest average inclination for udder floor, but the daughters of sire 5 had the lowest coefficient of variation and the daughters of sire 2 had the highest coefficient of variability for this character. Perhaps these are additional indications that the slope of rump and slope of udder floor are inherited independently of each other.

In view of the history of the daughters of sire 3 at the Woodward Station, it is surprising that his daughters do not show a greater slope of the udder floor, and an even greater coefficient of variability for this character and for milk yield and butterfat yield. The daughters of this sire suffered rather uniformly at calving time from a severe inflammation of the udder and an edematous condition that persisted long after calving. It was thought that the severity of this condition resulted in some breaking down of the udder and interference with the expression of their normal inherent producing ability.

SUMMARY AND CONCLUSIONS

1. Man's ideas of beauty in the bovine long ago insisted that the rump should be level, though most species of animals have sloping rumps. Breeders for many years have selected for level rumps. But the inheritance for the sloping rump still persists, and is continually occurring in all breeds of dairy cattle, though with decreasing frequency. Along with the selection of breeding animals for level rumps, there have been developed over a period of years opinions that there are certain associated defects that are likely to be inherited along with the sloping rump. Of these opinions the most common and the most important were: (a) that the floor of the udder would parallel the rump—that with a sloping rump there would be a tilted udder; (b) the tilted or sloping udder would be deficient in the front quarters and would, therefore, have less capacity; (c) that difficult calving and lower breeding efficiency occurs in cows with sloping rumps.

2. In the experimental breeding herds of the Bureau of Dairy Industry animals were photographed once a year under carefully controlled conditions as to posing on a level track with camera on a fixed post at a uniform distance from animal and at uniform height from ground, and with the same camera used for all the pictures. It was found that these pictures could be satisfactorily used in obtaining the degree of slope of the rump; they can also be used for determining the degree of slope of the udder, though with somewhat less accuracy than that of the rump. The degree of accuracy of the slope obtained from the photographs was checked with a clinometer on the living animal and found to be satisfactory.

3. The material for this paper was obtained from a series of more than 500 photographs of 155 females in the experimental breeding herd of Holstein-Friesian cattle at the United States Experiment Station at Woodward, Okla.

4. The animals with the straightest rumps were in the age group of 1 to 6 months. From that age the slope of the rump became progressively greater up to the age of 5 years. The most rapid change in the slope of rump came at the younger ages. In one group the per cent increase in degree of inclination of the rump from the age group of 1–6 months to the age group of 7–18 months was 13.8; from 7–18 months to 19–30 months the degree of inclination was 12.1 per cent greater, and from 19–30 to 31–42 months, the increase was 5.8 per cent.

5. The increase in degree of udder slope with advance in age was more pronounced than the change in rump slope. The maximum slope of udder was at 79–90 months of age, and the per cent increase in the degree of slope at this age was 88.1 per cent greater than the degree of slope in the 19–30 month age group. As with the rump, the greatest increase in the slope of

the udder came in the younger age groups with decreasing increments with advance in age.

6. The correlation coefficient for the relationship of the degree of inclination of the rump to the degree of inclination of the udder for 87 animals was + 0.021 ± 0.11; the correlation coefficient for relation of degree of sloping rump to producing ability was - 0.013 ± 0.107; the correlation coefficient for relation of degree of inclination of the udder to producing ability was - 0.196 ± 0.103. None of these coefficients was significant.

7. Herd records were not sufficiently complete to determine the relationship of the sloping rump to difficult calving. A correlation coefficient on the relation of the degree of inclination of the rump to the number of services per conception was + 0.220 ± 0.088. While hardly significant, this coefficient does show a more pronounced trend than the other three coefficients.

8. Study of the data on the daughters of the different sires used in the experiment indicates that the sloping rump and the tilted udder are inherited characters, probably of a multiple factor or blending type, and that the slope of the rump and the tilt of the udder are inherited independently of each other. The average of the actual slope of either the rump or the udder of several full sisters fits quite closely the expected slope calculated from the equal parent index of all the daughter-dam pairs for the sire and the actual slope of the dam.

9. Variability was decreased in the slope of rump and in the slope of the udders of the inbred daughters of one sire while the variability in milk and butterfat yields was increased. In a second sire the inbred daughters had a lower variability than the outbred daughters in slope of rump, slope of udder, milk yield, and butterfat yield, though the number of inbred daughters was rather small. The degree of variability was not associated with the degree of the slope of either the rump or the udder.

CAN GOOD PRODUCING COWS BE FED IN SUCH MANNER AS TO MAINTAIN THEIR WEIGHT?

R. R. GRAVES[1]

Students of dairy cattle nutrition often say that dairy cows should be so fed that they will not lose weight; that if a good producing cow is losing weight she is not receiving enough nutrients. If she is producing well and is not gaining or losing in weight she is correctly fed. In other words the weight of the cow during the lactation period is one of the best guides to good feeding.

A few years ago we conducted feeding experiments at field stations to determine the relative production of cows when on limited grain rations and on rations of roughage alone and when they were receiving what we termed a full grain ration, that is when they were fed grain at the rate of 1 pound to each 3 pounds of milk produced. It was noted that the cows on roughage alone lost weight quite rapidly early in the lactation period and then gained slowly during the remainder of the period. This indicated, apparently, that the cows were unable to eat enough of the bulky roughage ration to meet their requirements for production and maintenance in the early months of lactation. This is probably the reason why these cows did not approach more closely, when on a good quality of alfalfa hay, their level of production when receiving the full grain ration, rather than because of any deficiencies in the hay.

In one experiment 11 cows were fed throughout a 12-month lactation period on a ration consisting entirely of alfalfa hay, and 8 of these cows were fed through a second successive lactation period on alfalfa hay alone.[2] In the first lactation period these 11 cows lost in the first month an average of 107 pounds, in the second month 43 pounds and in the third month 7 pounds; in the fourth month they gained 4 pounds, in the fifth month they lost 8 pounds, and thereafter they gained in weight each month. Their total average loss, from their "after-calving" weight, during 4 of the first 5 months of the lactation period was 165 pounds, and their total average gains during the remaining 8 months was 122 pounds; therefore, in the twelfth month they had failed to regain their after-calving weight by 43 pounds.

The 8 cows that completed a second successive lactation period on alfalfa hay alone lost weight only in the first 2 months of lactation—an average of 91 pounds for the 8, and during the remainder of the lactation period gained

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[2] Feeding Dairy Cows on Alfalfa Hay Alone. U.S.D.A. Tech. Bul. 610, March, 1938.

123 pounds, so that they weighed an average of 33 pounds more in the twelfth month than their after-calving weight.

In an experiment at the Utah Station[3] 12 cows were fed through three successive 10-month lactation periods, in one of which they were fed only alfalfa hay and pasturage in season; in a second alfalfa hay, and ground barley fed at the rate of 1 pound to each 5.7 pounds of milk produced, plus pasturage in season; and in a third alfalfa hay and corn silage and pasturage in season. The weights of these 12 cows followed a very similar pattern during the three lactation periods. In the first they lost 104 pounds the first month and 17 pounds the second month—a total average loss of 121 pounds in 60 days. They gained it all back in the next 8 months and were 10 pounds over the after-calving weight in the tenth month. In the second lactation period, the one in which they received ground barley in addition to alfalfa hay, they lost an average of 105 pounds the first month, 14 pounds the second, and 4 pounds the third month, a total shrinkage of 123 pounds in the first 3 months of lactation. In the next 7 months their total average gain was 104 pounds so that in the tenth month of lactation they lacked 19 pounds of regaining their after-calving weight. In the third lactation period, when they received alfalfa hay and corn silage, the average loss of the 12 cows was 105 pounds the first month and 15 pounds the second month—a total loss of 120 pounds. Starting with the third month they made some gain each month, making a total average gain at the end of the tenth month of 154 pounds and putting them 34 pounds over their after-calving weight. Such uniformity of losses and gains for 12 cows over three lactation periods is quite remarkable.

So far as we could determine these severe losses in weight caused no physical injury to these cows, but it was no doubt associated with a lower level of production than would have been the case had they been fed in such manner as to enable them to maintain a more uniform weight. But can good dairy cows be fed so that there will be little change in their weights during the lactation period? A study was made of the changes in weight through a 12-month lactation period of 20 2-year-old Jerseys on Register of Merit test at the Lewisburg, Tenn., Field Station, of 20 2-year-old Holstein heifers on Advanced Registry test at the Huntley, Mont., Field Station, of 9 2-year-old Holsteins and 9 mature Holsteins on Advanced Registry test at the Beltsville Station, and of 9 2-year-old Jerseys and 9 mature Jerseys on Register of Merit test at the same station.

At the Lewisburg Station cows on Register of Merit test are fed legume hay *ad lib*, and a grain mixture fed at the rate of 1 pound to each 3 pounds of milk produced. They are also on pasture during the pasture season. At the Huntley Station cows are fed alfalfa hay *ad lib*, corn silage, and a grain mixture at the rate of 1 to 3. They are not pastured. At the Beltsville

[3] U.S.D.A. Tech. Bul. 724, April, 1940.

Station the method of feeding is somewhat different. The requirements of the cows for maintenance and production are calculated every 10 days. After allowing for the alfalfa hay and corn silage consumed, enough of a grain mixture is fed to provide total digestible nutrients 10 per cent in excess of the requirements. Some heavy-producing cows will not consume enough feed early in their lactation periods to provide the 10 per cent over requirements, but they were fed as closely to that level as their consumption permits. This no doubt results in somewhat more liberal feeding than is the case at the Lewisburg and Huntley Stations.

THE JERSEYS

The Beltsville 2-year-old Jerseys had an average weight of 868 pounds 13 days before calving, the individual weights ranging from 749 to 1,010 pounds. Their average weight 11 days after calving was 803 pounds, ranging from 695 to 900 pounds. The loss in weight from the before-calving to the after-calving was approximately 8 per cent of the latter weight. In the tenth month of lactation all but one of the 9 2-year-olds had reached a weight that exceeded her pre-calving weight. The one exception had the heaviest before-calving weight.

In the twelfth month of lactation they had reached an average weight of 951 pounds, a gain of 148 pounds over the after-calving weight, or 18.4 per cent. The lowest gain was 109 pounds and the highest gain was 214 pounds.

These 9 2-year-old Jerseys were evidently able to get on full feed quickly for four of them had no monthly weight that was below their after-calving weights and two more had monthly weights that were only 2 pounds and 7 pounds below their respective after-calving weights. The remaining 2 reached low weights that were 19 pounds and 42 pounds below their respective after-calving weights. They were one pound below the average after-calving weight in the first month of lactation but thereafter they made a gain each month.

Three of these heifers did not conceive during the lactation period, but they did not affect the result since their average gain from the after-calving weight to the twelfth month weight was 140 pounds, while the gain for the other 6 heifers was 153 pounds and they carried their calves an average of 188 days during the lactation period.

Feeding nutrients at the rate of 10 per cent in excess of requirements enabled them to gain consistently through the lactation period, to exceed their after-calving weights by an average of 148 pounds and to produce at the average rate of 8,994 pounds of milk, 479 pounds butterfat, starting at the age of 2 years, 1 month.

The 20 2-year-old Jersey heifers at the Lewisburg, Tenn., Station had an average before-calving weight of 769 pounds—99 pounds lighter than the before-calving weights of the 9 2-year-old Jersey heifers at Beltsville.

They ranged from 630 to 890 pounds. Their average after-calving weight was 729 pounds, with a range from 560 to 830 pounds. Thus the Lewisburg 2-year-olds were 12.8 per cent lighter than the Beltsville Jerseys before calving, and 10 per cent lighter after calving. The Lewisburg 2-year-olds lost only 40 pounds from the before-calving weight to the after-calving weight—equivalent to 5.5 per cent of the latter. The Beltsville Jersey shrinkage was 8 per cent. Only 12 of the 20 reached their before-calving weights in the tenth month of lactation.

In the twelfth month their average weight was 836 pounds and the average gain over the after-calving weight was 118.7 pounds or 16 per cent.

They lost very slightly in the first, second, fourth and sixth months—the total average loss was only 21 pounds. Actually they just about held their weight during the first 6 months, the total average gains of 20 pounds balancing the total average loss of 21 pounds, but starting with the seventh month they gained consistently during the remainder of the lactation period. This in contrast with the Beltsville 2-year-old Jerseys that gained from the first month to the twelfth.

The Lewisburg heifers were experiencing breeding trouble. Only half of them conceived during the lactation period, but this does not appear to have materially affected the results so far as gains are concerned. The 10 heifers that did not conceive made an average gain from after calving to the twelfth month of 118 pounds, while the 10 heifers that carried calves an average of 176 days during the lactation period made average gains of 122 pounds. The average butterfat yield was not materially affected by pregnancy; the 10 that carried calves an average of 176 days had an average yield of butterfat of 393.6 pounds and those that did not conceive had an average yield of 385.2 pounds.

The weights of the mature Beltsville Jerseys behaved in a somewhat different manner than did those of the 2-year-olds. The mature cows had an average before-calving weight of 1,201 pounds, 79 pounds or approximately 7 per cent greater than their after-calving weight. This is only 1 per cent less than the shrinkage of the 2-year-olds. Only four cows attained their precalving weights in the tenth month. In the twelfth month all had passed their after-calving weights, but their gain was only 6.6 per cent of the after-calving weight, approximately one third the percentage gain made by the Beltsville 2-year-olds. Their losses in weight during the lactation period were more pronounced than those of the 2-year-olds. All but two had lower weights than the after-calving weight during the lactation period. The low points were very scattered, two of them occurring as late as the eighth and the ninth months of the lactation period. After the first month the Beltsville 2-year-olds showed an average gain each month, while the mature Jerseys, with the exception of the second month, when they showed a loss, just about held the average after-calving weight through the seventh month of lactation. Starting with the eighth month they showed a material gain each month.

THE HOLSTEINS

The average before-calving weight of the Beltsville 2-year-old Holsteins, 1,238 pounds, was almost 12 per cent greater than their after-calving average. Like the 2-year-old Jerseys they made a gain in weight each month, excepting the first, which was the same as the after-calving weight. Only three of them dropped below their after-calving weight at any point in the lactation period. Three of them failed to attain their before-calving weights in the tenth month, and all of them passed their after-calving weights by substantial margins by the twelfth month of lactation. These gains ranged from 95 to 367 pounds, and the average gain of 234 pounds was 21 per cent of the average after-calving weight. This is a larger percentage gain than that made by the Beltsville 2-year-old Jerseys.

Twenty 2-year-old Holstein heifers at the Huntley, Mont., Station had an average before-calving weight of 1,279 pounds, 13.7 per cent greater than their after-calving average. Both the before-calving and the after-calving average weights were somewhat higher than those of the Beltsville 2-year-olds. However, the system of feeding at Huntley did not enable them to gain as consistently during the lactation period as did the 2-year-olds at Beltsville under the system of feeding prevailing at that station. Only 5 of the 12 heifers attained their before-calving weights in the tenth month of lactation, and 17 of the 20 fell below their after-calving weights during the lactation period. On the average the Huntley heifers were below their after-calving weight for the first three months of the lactation. The low weight during the lactation for the 17 heifers showing a loss ranged from 15 to 220 pounds. However, by the twelfth month they had all passed their after-calving weights, the average gain at that time being 180.5 pounds or 16 per cent of the after-calving average. This is less than the percentage gain of the Beltsville 2-year-olds by some 5 per cent. Actually the Huntley heifers made an average total gain during the lactation period of 215 pounds, as compared to an average gain of 234 pounds by the Beltsville 2-year-olds, but the Huntley heifers had an average loss of 30 pounds in the first month to overcome, while the Beltsville heifers had no loss. The Huntley heifers were heavier than the Beltsville heifers at the start of the lactation period by an average of 19 pounds, but they were lighter in the twelfth month of lactation by an average of 28 pounds.

The mature Holstein cows at Beltsville had an average weight before-calving of 1,719 pounds, 8.4 per cent more than their after-calving weight. This is a smaller shrinkage due to calving than was experienced by the Holstein 2-year-olds at Beltsville and Huntley.

On the basis of averages these 9 mature cows remained remarkably close to their after-calving weight during the first, third, and fourth months of lactation. In the second month they dropped 15 pounds below their after-calving weight. From the fifth to the eighth months of lactation their average weights only ranged from 1,607 to 1,611 pounds, but during the remaining 4 months they made consistent gains each month. In the tenth month, only one cow had regained her before-calving weight. In the twelfth month all had gained over their after-calving weights, the average gain of 111 pounds being an increase of 7 per cent. Thus the per cent gain over the after-calving weight in the mature Holsteins was only about one third as great as for the 2-year-old Beltsville heifers. This is almost the same relationship as was shown by the relative gains of the mature Jersey cows to those of the 2-year-olds.

Seven of the 9 Holstein cows had low points in their weights that ranged from 2 to 103 pounds below their after-calving weights. Four of the 7 cows reached the lowest level of weight in the second month, one in the third month, one in the fourth and one in the seventh month of lactation.

CONCLUSIONS

1. The shrinkage from the before-calving to the after-calving weights, on the basis of the per cent of the after-calving weight, was greater in the Holsteins than in the Jerseys and greater in the 2-year-olds than in the mature cows. The greatest shrinkage was in the Huntley Holstein 2-year-olds—13.7 per cent—and the least in the Lewisburg Jersey 2-year-olds—5.5 per cent. The smaller Jersey calf weight accounts for a part of the difference between the Holstein and Jersey breeds.

2. In total average gains from the after-calving weight to the twelfth month of lactation, expressed as per cent of the after-calving weight, the Beltsville 2-year-old Jerseys and 2-year-old Holsteins gained 18.4 and 21 per cent, respectively, and the Beltsville mature Jersey cows and the Beltsville mature Holstein cows gained 6.6 and 7 per cent, respectively. These four groups were under the same system of feeding—requirements plus 10 per cent. It appears that the 2-year-olds—Jersey and Holsteins—made from 2.5 to 3 times the gains of the mature cows. The Lewisburg 2-year-old Jerseys and the Huntley 2-year-old Holsteins had the same average percentage gain—16 per cent. The system of feeding these two groups, while the usual full grain feeding system, is somewhat less liberal than the requirements plus 10 per cent.

3. Under the system of feeding followed at the Beltsville Station the average weights of both the 2-year-old Jerseys and the 2-year-old Holsteins were approximately the same in the first month of lactation as their after-calving weights, but thereafter they made gains each month. The gains were not as rapid in the early months as in the last months of the lactation period but on the average there were no losses. The mature Jerseys and the mature

Holsteins under the same system of feeding showed a different pattern of gains from those of the 2-year-olds but were remarkably similar to each other. The Jersey cows had an average loss of 78 pounds in the second month but gained it back in the third. In the first 7 months of lactation they lost 82 pounds and gained 90 pounds on the average, so that they were just about holding even. In the last 5 months they had an average gain of 68 pounds. The Holsteins had an average loss of 20 pounds in the second month. In the first 7 months they lost an average of 22 pounds and gained an average of 45, so they were a little more than holding even. In the last 5 months their average gain was 89 pounds. The Lewisburg 2-year-old Jerseys held an even weight the first 6 months and made consistent gains the last 6 months. The Huntley 2-year-old Holsteins had an average loss of 30 pounds the first month, and gained each month thereafter.

4. It appears that, with the requirements-plus-10-per-cent system of feeding, 2-year-olds will gain from the first month of lactation, and with mature cows the average gains will somewhat more than balance the losses in the first 7 months and rapid gains will occur during the balance of the lactation. Under the usual full-grain system of feeding, that is 1 pound of grain to each 3 pounds of milk produced, the average losses and gains balanced each other in the first 6 months and consistent gains occurred during the balance of the lactation with 2-year-old Jerseys, and with 2-year-old Holsteins after an average loss the first month, there were gains on the average each month during the balance of the lactation period.

FROZEN HOMOGENIZED MILK. II. EFFECT OF FREEZING AND STORAGE TEMPERATURES ON THE CHEMICAL AND BACTERIOLOGICAL PROPERTIES OF HOMOGENIZED MILK

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In part I, "Effect of Freezing and Storage Temperature on the Physical Characteristics of Homogenized Milk" (1), it was shown that freezing and storage temperatures affect the physical character of homogenized milk. To determine if these conditions cause chemical changes which may contribute to the physical changes, a study was made of the shift of the chemical constituents in samples of homogenized milk frozen and stored under the same conditions as the samples used to study the physical changes. Bacterial counts were also made on a number of the samples to determine whether biological changes occur in frozen homogenized milk.

In a study on frozen homogenized milk, Cvitl (2) analyzed the outer layer, the part which froze first, as well as the top, middle and bottom portions of the remainder of the sample and found that the central portion was richer in fat, casein, albumin, globulin, sugar, and chloride ion than the upper or lower portions and that the outer layer was the poorest in these constituents. Baldwin and Doan (3) reported that when milk, whose creaming ability was destroyed by heat or homogenization, was frozen, the fat concentration of the unfrozen portion increased progressively with the extent of freezing, while that of the frozen portion decreased at first, but finally approached the fat percentage of the original milk as the extent of freezing approached 100 per cent. Trout (4) reported that when homogenized milk was frozen and then thawed a marked settling of the fat and solids-not-fat was noted. The lower 15 per cent of creaming cylinders of slowly thawed frozen homogenized milk contained as high as 7.7 per cent fat and 24.60 per cent total solids in contrast with 2.0 per cent and 5.50 per cent, respectively, in the upper 15 per cent layer. Fabian and Trout (5) found that from a bacteriological standpoint there is no reason why clean, wholesome, fresh cream cannot be pasteurized and stored for a period of 1 year in glass, paper, or tin containers at temperatures ranging from – 5° to – 10° F.

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PROCEDURE

Sections of the homogenized milk were obtained for analysis by dividing the quart samples, while frozen, either into thirds, or into halves.

The following methods of analysis were used:

a. Protein—Kjeldahl-Gunning-Arnold Method using the factor of 6.38.
b. Fat—Mojonnier Method.
c. Total solids—A.O.A.C. Method.
d. Lactose—Munson-Walker Method.
e. Chloride ion—Volhard Method using the milliequivalent value of 0.00355.
f. Ash—Heated in a muffle furnace at a temperature not higher than 525° C.
g. Bacteria—The bacteria content of the milk was determined by the standard plate count in accordance with the method as outlined in Standard Methods for the Examination of Dairy Products, APHA, 8th edition, 1941. The plates were incubated at 37° C.

EXPERIMENTAL RESULTS

Chemical

The results of the chemical analyses of aliquots from different sections of quart samples of homogenized milk, frozen in paper containers of one quart capacity and stored under various conditions, are shown in table 1. The analyses of two samples of the homogenized milk prior to freezing gave the following average values: protein, 3.24 per cent; fat, 3.72 per cent; total solids, 12.26 per cent; lactose, 4.77 per cent; and chloride, 0.095 per cent.

Table 1 shows that when homogenized milk was frozen and stored in the frozen state the solid constituents were more concentrated in the bottom section. When the quart samples were divided into two equal parts the lower halves contained a larger proportion of the milk solids. Analyses of the frozen samples after divisions into three equal parts disclosed the fact that the highest concentration of milk solids was in the bottom third, the next highest concentration was in the middle third, and the lowest was in the top third. It may also be seen from table 1 that the analyses of the middle third were in good agreement with those of unfrozen milk, as well as samples of milk which had been frozen and stored and then thawed. Those slight discrepancies which occurred may be attributed to experimental error, especially in samples showing separation.

The data in table 1, A, were obtained by the analysis of milk samples frozen and held at – 10° C. (14° F.) for 21 to 100 days, at – 32.8° C. (– 27° F.) for 30 to 109 days, and at – 40° C. (– 40° F.) for 51 to 82 days. The analyses of the individual samples indicated that the freezing and storage temperatures as well as the storage time may influence the distribution of the milk solids between the various sections of the frozen milk.

TABLE 1

Chemical analyses of different sections of homogenized milk frozen in quart paper containers and stored under various conditions

No. samples	Section analyzed	% Protein	% Fat	% Total solids	% Lactose	% Chlorides	% Ash
A. Frozen and stored at constant temperature (–10° C., –32.8° C., –40° C.)							
8	Top third	2.55	2.77	9.85	3.85	0.077	0.58
	Middle third	3.17	3.48	12.27	4.81	0.095	0.74
	Bottom third	3.66	4.23	13.94	5.45	0.104	0.82
6	Top half	3.09	3.16	10.88	4.11	0.093	
	Bottom half	3.88	3.91	13.65	5.13	0.116	
11	Whole quart	3.35	3.64	12.66	4.79	0.096	0.73
B. Frozen and held at –32.8° C., or –10° C., followed by storage at a lower temperature (–40° C. or –32.8° C.)							
6	Top half	3.03	3.03	10.71	3.99	0.093	
	Bottom half	4.00	3.92	13.94	5.20	0.117	
C. Frozen and held at –40° C., –32.8° C., or –10° C., followed by storage at a higher temperature (–32.8° C., –10° C., or –3° C.)							
10	Top third	2.25	2.56	8.77	3.33	0.067	0.51
	Middle third	3.14	3.30	12.15	4.67	0.093	0.73
	Bottom third	3.98	4.04	15.17	6.07	0.115	0.90
6	Top half	3.16	3.33	11.21	4.14	0.093	
	Bottom half	3.80	3.93	13.51	4.99	0.117	
D. Frozen and held at –32.8° C. or –10° C., exposed to room temperature (23° C.) for ½ hour, then stored at –10° C.							
24	Top third	2.37	2.39	9.44	3.49	0.070	0.53
	Middle third	3.15	3.14	12.06	4.61	0.094	0.69
	Bottom third	3.75	3.74	14.24	5.56	0.107	0.81
2	Top half	2.95	2.99	10.46	4.00	0.091	
	Bottom half	3.97	3.94	13.91	5.35	0.117	
E. Frozen and held at –32.8° C. or –10° C., exposed to room temperature (23° C.) for 1 hour, then stored at –10° C.							
24	Top third	2.53	2.52	9.97	3.84	0.076	0.61
	Middle third	3.08	2.71	12.33	4.83	0.095	0.73
	Bottom third	3.36	3.42	13.58	5.15	0.099	0.78
2	Top half	3.04	3.13	10.98	4.20	0.095	
	Bottom half	3.84	3.80	13.38	5.01	0.112	
F. Frozen and held at –32.8° C. or –10° C., exposed to room temperature (23° C.) for 2 hours, then stored at –10° C.							
24	Top third	2.51	2.61	9.82	3.64	0.070	0.54
	Middle third	2.83	2.87	12.04	4.62	0.093	0.67
	Bottom third	3.66	3.40	14.12	5.47	0.106	0.82
2	Top half	2.87	2.90	10.23	3.85	0.088	
	Bottom half	3.97	3.87	13.96	5.29	0.120	
G. Frozen and held at –32.8° C. or –10° C., exposed to room temperature (23° C.) for 4 hours, then stored at –10° C.							
24	Top third	1.83	2.09	7.58	2.72	0.054	0.50
	Middle third	2.81	2.75	11.42	4.33	0.084	0.70
	Bottom third	4.47	3.92	17.69	7.01	0.136	0.99
2	Top half	1.83	1.88	6.56	2.40	0.056	
	Bottom half	4.93	4.58	17.32	6.57	0.145	

Table 1, B, presents the data obtained when two samples of homogenized milk were frozen at – 32.8° C. (– 27° F.) then moved to – 40° C. (– 40° F.); two frozen at – 10° C. (14° F.) then moved to – 32.8° C.; and two frozen at – 10° C. then moved to – 40° C. The milk was held at the initial freezing temperature for 9 days and at the lower temperature for 54 to 80 days prior to analysis. The results given in table 1, A and B, show that lowering the storage temperature of frozen homogenized milk did not significantly affect the distribution of milk solids.

As indicated in table 1, C, some of the samples were frozen at – 40° C. (– 40° F.) and moved to – 32.8° C. (– 27° F.) or to – 10° C. (14° F.); some were frozen at – 32.8° C. then moved to – 10° C. or to – 3° C. (26.6° F.); and others were frozen at – 10° C. and moved to – 3° C. The samples were held for an average of 3 days at the lower temperature and for an average of 70 days at the higher temperature prior to analysis. A comparison of the results in C with those in A and B (table 1) indicates that moving frozen milk to a higher storage temperature does not materially affect the results of the chemical analyses of the different sections of the sample.

The data presented in table 1, E, F, G, and H, were obtained by the analysis of samples which were exposed to room temperature for various

TABLE 2

Effect of freezing and storage temperatures on the standard plate count of homogenized milk

No. samples	Average storage time (days)	Average standard plate count per ml.
A. Frozen and held at constant temperature (– 40° C., – 33° C., or – 10° C.)		
3	56	11,000
3	88	12,000
B. Frozen and stored at a constant temperature (– 10° C.) then moved to a lower temperature (– 33° C.)		
1	59	15,000
1	92	20,000
C. Frozen and stored at a constant temperature (– 33° C. or – 40° C.) and then moved to a higher temperature (– 10° C. or – 33° C.)		
3	53	2,000
3	85	3,000
D. Frozen and stored at a constant temperature (– 33° C. or – 10° C.) exposed to room temperature, then stored at – 10° C.		
2 (exposed ½ hr.)	59	4,000
2 (exposed ½ hr.)	92	7,000
2 (exposed 1 hr.)	59	3,000
2 (exposed 1 hr.)	92	4,000
2 (exposed 2 hrs.)	59	3,000
2 (exposed 2 hrs.)	92	6,000
2 (exposed 4 hrs.)	59	3,000
2 (exposed 4 hrs.)	92	6,000

lengths of time between storage at two different freezing temperatures. These conditions were intended to simulate those which occur when frozen milk is moved from storage to a freezer on a ship. A comparison of the results shown in E, F, G, and H with each other and with those in C demonstrates that such conditions did not affect the distribution of milk solids unless the frozen milk was exposed to room temperature for four hours. After four hours there was a considerable increase in the concentration of milk solids in the lower section and a corresponding decrease in the upper section.

Bacteriological

Standard plate counts were performed on samples of milk prior to freezing and again after freezing and storage under different conditions in order to determine whether these environments permit multiplication of bacteria. The average plate count of the milk prior to freezing was 28,000 per ml. Table 2 shows the average plate count after the milk had been frozen and stored.

The data in table 2 indicate that freezing milk and storing it in the frozen state had a tendency to lower the number of bacteria per milliliter as determined by the standard plate count and further that the bacterial content was not materially affected by changes in storage time and temperature, or by exposure to room temperature for four hours.

Results obtained on individual samples indicated that the count was not influenced by the freezing or storage temperatures.

SUMMARY

It has been found that when homogenized milk is frozen the solid components tend to concentrate in the lower portions of the sample. This distribution was not materially affected by changes in freezing and storage temperature. Exposure of the frozen milk to room temperature (23° C.) less than four hours did not alter the distribution of the milk solids, but at that time there was a significant shift of the solid components toward the lower portion.

Changes in the temperature at which frozen homogenized milk was stored did not materially affect the chemical composition of the different sections of the quart samples.

The chemical analyses of the various sections of frozen homogenized milk were not affected by exposure to room temperature, 23° C. (73.8° F.), unless the milk was exposed for four hours. When the milk was exposed for four hours there was a considerable increase in the percentage of milk solids in the bottom third and a considerable decrease in the percentage of the constituents in the top third of quart samples.

Homogenized milk that was frozen and subsequently thawed at room temperature (23° C.) changed in physical character. The degree of change was dependent upon the freezing and storage temperature and the length of storage. However, these conditions did not cause any significant change in the chemical character of the milk, indicating that the chemical character of frozen homogenized milk does not contribute to the physical changes.

Freezing and storing homogenized milk in the frozen state had a tendency to lower the number of bacteria per milliliter as determined by the standard plate count. This decrease was not materially affected by freezing and storage temperatures, by changes in the storage temperature, or by exposure to room temperature for four hours.

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TOXICITY OF PHENOTHIAZINE DERIVATIVES EXCRETED IN THE MILK OF DAIRY COWS TREATED WITH MASSIVE DOSES OF THE DRUG*

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The effectiveness of phenothiazine as an anthelmintic has resulted in its widespread and, in some cases, indiscriminate administration to animals. In recent years the recognition of the toxic reactions of this drug has led to increased caution in its dosage. Stewart (8), in a review of the subject, indicated that man and the bovine are the most susceptible species to phenothiazine poisoning, and that the young are less resistant than the adults. Hence, it would appear that the infant might be highly sensitive to this drug.

Portions of phenothiazine derivatives are excreted in the milk of lactating ewes (9) and goats (6) following medication. Though milk contaminated with these derivatives usually is diverted from food channels, occasionally it is offered inadvertently for human consumption (1), thus constituting a potential health hazard.

Adult cattle ordinarily are not heavily infested with internal parasites, but the unthrifty condition of a cow in areas where parasitic infestation is common may lead to phenothiazine therapy. The possibility that milk from a cow treated with this drug might be fed to infants presented a problem warranting investigation. In this study single massive doses of phenothiazine were given to individual lactating cows for the purpose of ascertaining: *the clinical effects on the cows, the period of elimination of the phenothiazine derivatives in the milk, and the toxic effects of the contaminated milk on young rats.*

EXPERIMENTAL

Effects of massive doses of phenothiazine on lactating dairy cows. A representative of each of three breeds, Guernsey, Holstein, and Jersey, were used in this investigation. Pertinent data on the experimental subjects are presented in table 1.

The cows were in an excellent state of health. They were subjected to standard managerial and feeding practices; the rations, consisting of a

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TABLE 1

Experimental cows that received massive doses of phenothiazine

Breed	Age	Body weight	Stage of gestation	Stage of lactation
	years	*lbs.*	*days*	*days*
Guernsey	7	1055	78	210
Holstein	5	1065	154	182
Jersey	8	990	51	174

concentrate mixture, beet pulp and either hay or silage, were fed twice daily, at the milking periods.

Each cow was dosed with 125 grams of commercial phenothiazine, which amount was approximately twice the maximum recommended for adult cattle. Observations during the four days following medication revealed no changes except a precipitous drop in the milk production of two of the cows and a slight decrease in that of the third. The milk yields persisted at the lowered level for two to three days but subsequently returned to the pre-treatment level for the Holstein and the Jersey cows, the higher producers, but remained low for the Guernsey.

Abnormal red coloration was observed on the udders of the cows the day following the administration of the drug. This, presumably, was the result of oxidation of the phenothiazine conjugates (5, 9) in the urine that had come in contact with the surface of the udder.

Period of excretion of phenothiazine derivatives in the milk. In accordance with common practices the cows were milked at 12-hour intervals. Samples of milk from the individual animals were collected at each milking for a period of 72 hours following administration of the drug.

Determinations of the presence of phenothiazine derivatives were made by qualitative methods described by Collier (5). Acidification of the milk samples with strong hydrochloric acid, producing a mauve color, was the most satisfactory test used. Exposure of the samples to air and light for several hours resulted in the development of a pink color in the serum phase of the milk. This color was difficult to detect in samples having a high carotenoid content; the yellow seemed to mask the pink. In addition to the procedures indicated by Collier (5), it was found that mixing "Aerosol",

TABLE 2

Period of elimination of phenothiazine derivatives in milk following medication

Breed	Hours after dosing					
	12	24	36	48	60	72
Guernsey	+++	+++	++	+	+	–
Holstein ..	+++	++	+	–	–	–
Jersey	+++	+++	++	+	+	–

a surface-tension-reducing reagent, with milk containing the conjugates of phenothiazine produced a transitory pink color, which was adequate for detection but unsatisfactory for estimating the degree of concentration.

As shown in table 2, the phenothiazine products were in the milk for periods of 36 to 60 hours after dosing. The highest concentrations apparently were in the first milking following administration.

Toxicity of phenothiazine derivatives excreted in the milk of the cows. Samples of milk collected from the individual cows at the 12-hour and the 24-hour periods were fed to young rats to ascertain whether any toxic reactions would be evinced. Immediately after the collections, half of each sample was stored in the raw state at 35° F., but the other half was boiled for three to five minutes (recommended treatment of milk for infant feeding) before storing.

Thirteen month-old rats, grouped as indicated in table 3, were restricted to diets of the various milk samples for 72 hours. During a preliminary adjustment period of 60 hours, the individual rats were fed pasteurized whole milk *ad lib.* The pasteurized milk diet was replaced by the experimental samples, a fresh supply being provided twice daily.

TABLE 3

Rates at which young rats consumed various samples of milk containing phenothiazine derivatives

Samples of milk fed		No. of rats	Av. wt. of rats	Daily consumption per 100 grams of body wt.	
Period of collection	Treatment			Average	Range
			grams	*cc.*	*cc.*
12-hour	None	3	55	70	63–78
	Boiled	3	55	74	72–79
24 hour	None	3	52	74	71–80
	Boiled	3	55	71	63–83
Daily (herd)	Pasteurized	1	57	77	

The rate of consumption of the milk samples, as shown in table 3, reveals a marked individual variation but no significant group differences. Evidently the presence of the phenothiazine conjugates in the milk did not change its palatability. Clinical examinations of the rats during and after the milk feeding period revealed no discernible toxic effects.

DISCUSSION

In accord with the report of Britton (4), the phenothiazine derivatives eliminated in the milk seemed to have a preservative value. The milk samples containing the conjugates were in excellent condition after storage for 31 days at 35° F. Furthermore, as noted by Swales and Collier (9), samples exposed to light and air in a warm room for several days showed no evidence of decomposition. These properties, either bacteriostatic or bactericidal, have been ascribed to the oxidation product, thionol (4). The microbiological phase of the subject merits further investigation.

Boiling the milk for several minutes apparently modified neither its physiological effects on rats nor its qualitative reactions to various agents. The effects of extensive boiling, producing a concentration of the products, were not investigated. This type of treatment, however, is beyond the limits of practical measures used in the home preparation of milk for direct consumption.

Although phenothiazine derivatives are eliminated in the milk of cows treated with the drug, the concentration of these conjugates from recommended therapeutic doses probably is too small to cause serious toxic reactions in the consumer. No ill effects have been demonstrated in pigs (2), kids (6) and lambs (9) that have consumed milk from their respective dams treated with the commonly prescribed doses of phenothiazine. In recovery trials with sheep Swales and Collier (9) observed that between 80 and 85 per cent of a therapeutic dose of phenothiazine was eliminated in the feces and urine; thus only a small percentage could be excreted in the milk.

Though it is hazardous to attempt to translate experimental findings, particularly in toxicity studies, from young rats to infants, the results of this investigation suggest that milk from cows given a therapeutic dose of phenothiazine is not likely to be toxic to the normal human subject. The cows in this experiment were given twice the recommended amount, which should have produced a concentration of derivatives in the milk at least as great as the maximum from the standard doses given to any other adult cattle. Furthermore, the rats, though more resistant than the human being (8), consumed quantities of milk per unit of weight from five to six times greater than normally would be fed to babies (7).

The element of human variation in susceptibility to phenothiazine, as indicated in results reported by Bercovitz *et al.* (3), warrants adherence to the general recommendation that milk from cows treated with the drug either be discarded or be used for purposes other than human consumption during the period that the derivatives are excreted. This introduces the problem of determining whether or not the conjugates are in the milk. Since the pink color that develops upon exposure to air frequently is difficult to detect in milk having highly pigmented fats, this procedure, though practical, cannot be regarded as an infallible indicator of the absence of the derivatives. Therefore, as a precautionary measure, it probably is well to divert the milk from food channels for a period of two to three days following medication.

SUMMARY

1. Oral administration of 125 grams of commercial phenothiazine, over twice the maximum recommended therapeutic dose, to healthy adult lactat-

ing cows produced no detectable deleterious effects other than a temporary repression of milk production.

2. Derivatives of phenothiazine were detected in the milk for periods of 36 to 60 hours following administration of the drug.

3. Young rats restricted to diets of the milk collected at the 12-hour and the 24-hour periods after dosage manifested no discernible symptoms of toxicity.

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A DEVICE TO AID IN DETERMINING THE EFFECTIVENESS OF DAIRY DETERGENTS

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A wide variety of water types is found in Florida. Because of the widespread limestone deposits, waters of widely varying degrees of hardness are encountered. Also, because of the proximity of the Atlantic Ocean and the Gulf of Mexico considerable concentrations of salt are found in the water in certain areas. A project is in progress to determine the most suitable washing powders to use with the various types of water. It is not the purpose of this paper to report the results of the experiments on washing powders but to describe the apparatus being used to aid in determining the effectiveness of various washing powders in cleaning dairy equipment.

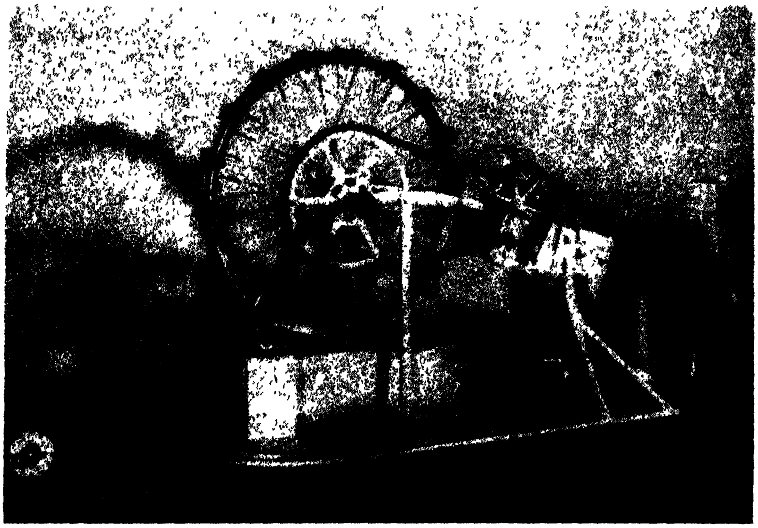

FIG. 1. Deterg-O-Meter—An apparatus designed to test the efficiency of dairy cleaners. Glass slides with a baked-on milk film are fastened onto the rim of the wheel by means of metal clips. The wheel is then lowered into water at 50° C. containing the washing powder to be tested. The wheel turns slowly, dipping the slides into the water and then rubbing them against the sponge rubber brush. The average number of revolutions required to clean the slides gives an index of the efficiency of the cleaner.

The wide scope of this problem suggested the need for a mechanical device to aid in making washing trials. Since no such device was available, one was built. It has been named the Deterg-O-Meter. It consists of a 16-inch metal bicycle wheel mounted in a pivoted frame which permits vertical movement, powered by an electric motor and geared to turn at 7 r.p.m. Evenly

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TABLE 1

Method of determining the "soil removal index"

Revolutions	Trial number					
	1		2		3	
	Slide number		Slide number		Slide number	
	Washing powder No. 1					
40	1, 13	80	14	40	5, 6	80
50	2, 3, 4, 6, 8, 9, 10, 12, 16	450	1, 8, 9, 12, 17, 2, 10, 11	400	2, 4, 8, 9, 10, 11, 13	350
60	5, 11, 14, 17, 18	300	5, 6, 7, 13, 16, 18	360	1, 3, 12, 14, 16, 17, 18	420
70	7	70	3, 4	140	7	70
		900		940		920
	$\frac{900}{17} = 52.9$		$\frac{940}{17} = 55.3$		$\frac{920}{17} = 54.1$	
	$\frac{52.9 + 55.3 + 54.1}{3} = 54.1$ soil removal index					
	Washing powder No. 2					
20	18	20	1, 2, 7, 12, 13, 16	120	11, 12	40
30	1, 2, 5, 6, 7, 11, 12, 13, 14, 15, 16	330	8, 10, 11, 14, 17, 18	180	1, 3, 4, 6, 9, 13, 14, 15	240
40	3, 4, 9	120	4, 5, 6, 9	160	2, 5, 7, 10, 16, 17	240
50	8, 10	100	3, 15	100	18	50
60					8	60
		570		560		630
	$\frac{570}{17} = 33.5$		$\frac{560}{18} = 31.1$		$\frac{630}{18} = 35.0$	
	$\frac{33.5 + 31.1 + 35.0}{3} = 33.2$ soil removal index					
	Washing powder No. 3					
30	4	30	7	30		
40					12	40
50					13	50
60	9, 11	120	1, 2, 6, 12	240	2, 9, 11	180
70	5, 8, 10, 12, 18	350	9, 11, 13, 15	280	1, 3, 6, 10, 14, 17	420
80	2, 3, 7, 14, 17	400	5, 8, 10, 14, 17	400	15, 18	160
90	6, 13, 16	270	16, 18	180	4, 7	180
100	1	100			8	100
110			4	110		
120	15	120			5	120
130						
140			5	140		
		1390		1380		1250
	$\frac{1390}{18} = 77.2$		$\frac{1380}{18} = 76.6$		$\frac{1250}{17} = 73.5$	
	$\frac{77.2 + 76.6 + 73.5}{3} = 75.7$ soil removal index					

spaced around the rim of the wheel are 18 numbered metal clips, which hold securely 18, 3 by 1 inch glass microscope slides.

Milk films are prepared on one side of the clean slides by spreading 0.4 ml. of whole milk evenly and uniformly over a rectangular area ¾ by 1⅛ inches. The slides then are heated in an oven for 3 hours at 120° C. which produces a tough hard film which is very resistant to cleaning.

Four liters of the water to be tested containing a weighed amount of the washing powder to be tested are warmed to 50° C. and poured into the pan. The slides are slipped under the numbered clips and the wheel is lowered so that when it revolves it dips into the water. The temperature of the water is maintained at 50° C. by means of a hot plate under the water bath. Immediately after dipping into the water, the slides rub against the sponge rubber brush. As the slides become clean they are removed from the wheel and a record is made of the number of revolutions required to remove the film. The average number of revolutions required to clean the 18 slides is determined.

Second and third runs are made and if these 3 runs check within 5 revolutions, the average of the 3 runs (54 slides) is accepted as being accurate. If the first 3 trials fail to check within 5 revolutions, additional runs are made until satisfactory checks are obtained. The brush is made of sponge rubber refrigerator door gasket material and a new brush is installed after each 20 runs. The data shown in table 1 illustrate the procedure used to calculate the "soil removal index." Other tests also are being made to aid in determining which powders are most suitable for washing dairy equipment in the various types of Florida water.

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The Constitution and By-laws of the American Dairy Science Association provides for the publication of the names of the nominating committee in the January issue of the JOURNAL OF DAIRY SCIENCE. It is the duty of this Committee, of which **A. C. Ragsdale** is the present chairman, to send its report to the secretary not later than April 1. The secretary will then send out the ballots to secure a final vote before the annual meeting. The results of the election will be announced at the annual meeting.

As a mmber of the Association, it is your obligation to express your wishes for officers and directors to any member of this Committee. Two candidates for vice-president and four for directors will be nominated. The present vice-president automatically becomes president.

The Nominating Committee will give consideration to the wishes of the members as expressed by correspondence and also will consider additional candidates. They have been instructed to study the list of past officers and directors (see pages 803 and 804 of the August, 1943, Journal) to plan to secure good geographic distribution and to recognize the desirability of representation from all lines of activity of our members.

This change in the Constitution was made so that our Association could elect its officers in a democratic way. This can only be done, however, if our members are prompt in giving their opinions freely to the Nominating Committee.

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Committees made up of five members retire one member every third year, and at the two intervening years two men each are retired. Vacancies caused by the expiration of a term are filled by three-year appointments. All appointments expire immediately following the annual meeting in the year designated.

In addition to five committees made up of members entirely in the Extension Section there are three joint committees with the Production Section. These committees have three men represented from each section and each year one man retires from each sectional group. The chairman of these committees is selected jointly by the two section chairmen.

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THE THIAMINE, RIBOFLAVIN, NICOTINIC ACID AND PANTOTHENIC ACID CONTENTS OF MARE'S COLOSTRUM AND MILK AND ASCORBIC ACID CONTENT OF THE MILK

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The importance of colostrum to the new-born animal has been emphasized by various investigators, and it is generally believed that new-born animals having access to colostrum do better than those that are fed only milk. The work of Lundquist and Phillips (8) suggests that some of the B vitamins play an important role in the prevention of certain diseases of the new-born calf. The work of Gamble, Earle, and Howe (4) emphasizes the importance of the proteins of colostrum for foals. While there is extensive literature on the vitamin content of milk of various species, information on the various B vitamins in colostrum has become available but recently and then only for colostrum of the human (2, 14, 15) and the cow and ewe (10). These studies show that colostrum differs from milk in respect to various members of the B vitamin group.

The present study was undertaken to provide information on the thiamine, riboflavin, nicotinic acid, and pantothenic acid contents of mare's colostrum as compared with mare's milk. Information on the B vitamins of various species is of interest for comparative purposes and possibly in the preparation of substitutes for feeding orphaned foals.

Information on the ascorbic acid content of mare's milk is very limited, and the two values found in the literature differ by about ten-fold. Holmes and associates (6) recently reported ascorbic acid values for mare's milk ranging from 0.6 to 2.3 mg. per 100 ml., while the ascorbic acid values reported by Cimmino (1) ranged from 8.7 to 19.7 mg. per 100 ml. of milk. The magnitude of the differences in the reported ascorbic acid content of mare's milk is too great to be accounted for on the basis of diet or breed differences. Therefore, it seemed desirable to obtain additional information on the ascorbic acid content of mare's milk.

EXPERIMENTAL

The mares used for this study represented various breeds of light horses. No attempt was made to control the dietary regimen of the mares. All were

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fed essentially the same except for the amount of green feed, which depended on season and availability of pasturage. Since riboflavin (12) and possibly other (13) of the B vitamins are known dietary essentials, diet may be a factor in the concentration in the colostrum and milk of some of the vitamins studied.

The colostrum was collected within 12 hours after foaling. All the milk samples were from mares that had been nursing foals for 30 days or longer. The samples were collected into brown bottles, and precautions were taken against photochemical destruction of any of the vitamins.

Thiamine was determined by the thiochrome method of Hennessy (5). Riboflavin was determined by the microbiological method (16) on the autoclaved sample following filtration at a pH of approximately 4.6 to remove the proteins and foreign growth stimulants. Nicotinic acid was determined by the microbiological method of Krehl, Strong, and Elvehjem (7), with the slight modification devised by Pearson and Leucke (11) which was found to be satisfactory for colostrum and milk. Pantothenic acid was determined by the method of Neal and Strong (9), following the enzymatic liberation of the vitamin by takadiastase and papain. The Evelyn photoelectric method (3) described for urine was adapted to the determination of the reduced ascorbic acid in milk by precipitating the proteins with an equal volume of 10 per cent trichloroacetic acid.

RESULTS AND DISCUSSION

Samples of colostrum were collected from eight mares and samples of milk from fifteen mares. Milk samples were collected from all of the animals from which colostrum was obtained except for mare number 1. The values for thiamine, riboflavin, nicotinic acid, and pantothenic acid expressed in micrograms per 100 ml. of colostrum are shown in table 1, and the values for milk in table 2. The values for reduced ascorbic acid per 100 ml. of milk are shown in table 3. Some of the milk samples for the ascorbic acid studies were obtained from animals not appearing in tables 1 or 2. The mares used for the ascorbic acid studies have, therefore, been designated by letter in order to avoid confusion with animals in the first two tables.

Thiamine. The average thiamine content of mare's colostrum was 38 µg. per 100 ml. as compared with 16 µg. per 100 ml. of milk. The fact that the thiamine content of the milk is lower than the value for colostrum is in accord with observations for the cow and ewe (10). The average thiamine content of cow's colostrum is 62 µg. per 100 ml., and of the ewe 108 µg. per ml. as compared with 38 µg. per 100 ml. for mare's colostrum. The corresponding average values for milk of the respective species are 38, 60, and 16 µg. per 100 ml. The average thiamine value for mare's milk reported in this paper is somewhat lower than the values reported for four animals by Holmes *et al* (6).

TABLE 1

Thiamine, riboflavin, nicotinic acid, and pantothenic acid content of mare's colostrum
(Values in μg. per 100 ml.)

Mare number	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid
1	65	162	155	700
2	40	135	167	750
3	37	112	114	800
4	21	125	150	900
5	34	125	185	425
6	34	120	178	400
7	38	165	200	950
8	40	160	134	1050
Average	38	138	160	747

Riboflavin. The average riboflavin content of mare's colostrum was 138 μg. per 100 ml. as compared with 40 μg. per 100 ml. for the milk. The fact that mare's colostrum is much richer than the milk in riboflavin is in accord with the observations on the cow and ewe (10), as cow's colostrum contains more than three times as much riboflavin as does the milk, while in the case of the ewe the difference is still greater. It is also of interest that the riboflavin contents of the colostrum and milk of the mare are much lower than the values for the cow and ewe. Mare's colostrum contains an average of 138 μg. per 100 ml. as compared with 610 μg. per 100 ml. of cow's colostrum and 2008 μg. per 100 ml. of ewe's colostrum; the corresponding values for the milk are 40, 177, and 436 μg. per 100 ml.

Nicotinic acid. The average nicotinic acid content of mare's colostrum was 160 μg. per 100 ml. as compared with 58 μg. per 100 ml. of milk. The nicotinic acid values reported here for mare's milk are slightly lower than values reported previously for four mares (6). The nicotinic acid value for mare's colostrum is higher than the value that has been reported (10) for cow's colostrum, but not as high as the value for ewe's colostrum.

TABLE 2

Thiamine, riboflavin, nicotinic acid, and pantothenic acid content of mare's milk
(Values in μg. per 100 ml.)

Mare number	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid
2	22	37	50	400
3	12	36	40	250
4	22	45	55	300
7	26	36	68	330
8	10	42	60	350
9	10	38	70	350
10	12	47	60	510
11	10	30	50	390
12	12	30	50	230
13	18	70	60	225
14	20	32	70	315
15	15	40	70	320
Average . . .	16	40	58	331

Pantothenic acid. The pantothenic acid value of mare's colostrum is much higher than has been reported for the colostrum of other species. The average pantothenic acid content of mare's colostrum was 747 μg. per 100 ml. as compared with values of 224 and 262 μg. per 100 ml. for the colostrum of the cow and ewe, respectively. The average value of 331 μg. of pantothenic acid per 100 ml. of mare's milk is of the same order as values previously reported (6) for three animals. At present there is no physiological explanation for the high pantothenic acid level of mare's colostrum or for the fact that in this species the colostrum contains significantly more pantothenic acid than the milk, whereas cow's or ewe's milk contains more pantothenic acid than does the colostrum.

Ascorbic acid. The reduced ascorbic acid was determined on samples of milk collected from eight mares. These mares had been in milk for 2 or more months. From table 3 it will be seen that the values for reduced ascorbic acid ranged from 9.26 to 14.46 mg. per 100 ml. of milk, with an average of 11.83 mg. This figure is approximately ten times the value reported by Holmes and associates (6), but it agrees reasonably well with the values ranging from 8.7 to 19.7 mg. per 100 ml. of milk which were reported by Cimmino (1).

TABLE 3

The reduced ascorbic acid content of mare's milk

Mare number	mg./100 ml.
A	13.62
B	9.26
C	11.95
D	14.46
E	12.45
F	12.95
G	9.96
H	9.96
Average	11.83

As a check on our analytical procedure we determined the reduced ascorbic acid in samples of milk from eight cows. The values for cow's milk ranged from 1.3 to 2.2 mg. per 100 ml. These figures agree well with numerous values reported for cow's milk. Thus mare's milk contains about five times as much ascorbic acid as cow's milk.

SUMMARY

Studies were made of the thiamine, riboflavin, nicotinic acid, and pantothenic acid contents of mare's colostrum and milk and of the reduced ascorbic acid content of mare's milk.

Mare's colostrum was found to contain an average of 38 μg. of thiamine, 138 μg. of riboflavin, 160 μg. of nicotinic acid and 747 μg. of pantothenic acid per 100 ml. The corresponding values for mare's milk are 16 μg. of thiamine, 40 μg. of riboflavin, 50 μg. of nicotinic acid, and 331 μg. of panto-

thenic acid per 100 ml. Mare's colostrum contains significantly less thiamine and riboflavin and more pantothenic acid than cow's colostrum. Cow's milk is richer in each of the four vitamins than mare's milk.

The average value for reduced ascorbic acid for mare's milk was 11.8 mg. per 100 ml. This is approximately five times greater than the ascorbic acid values found in this laboratory and reported in the literature for cow's milk.

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A STUDY OF HEAT TOLERANCE IN JERSEY COWS

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In a previous article, the author (1) reported that there was a strong correlation between air temperature and body temperature of Jersey cows when the air temperatures were above 75° F., and that there was a wide variaton in the body reaction of different cows under the same conditions. Rhoad (4, 5) has found this to be true also for beef cattle and has proposed using the body temperature (converted to a heat-tolerance coefficient for convenience) under standardized conditions as one of the criteria for judging the suitability of cattle for areas having considerable periods of high air temperatures (2, 3). Seath and Miller (6) analyzed data on Jersey and Holstein cows in Louisiana and found that relative humidity plays a minor rôle in comparison to air temperature as a factor influencing body temperature, respiration rate, and pulse rate.

The author has not found, in the published data on the characteristics of the heat tolerance of individual animals, answers to the following questions: (1) Is the body temperature of the individual sufficiently stable from year to year to be a sound measure of the heat tolerance of the animal, or, in other words, is the heat-tolerance coefficient a fixed individual characteristic, such as fat percentage? (2) Do the age of the animal, the stage of lactation, and the stage of gestation affect the body reaction? (3) Are there real differences in reaction between individuals and groups of cows? These questions are of considerable importance to the livestock industry in the southern United States and in tropical countries, where the production of both beef and dairy products is low. This study was made in an attempt to answer them.

EXPERIMENTAL METHODS

The methods of gathering the data have been described in a previous report (1). For this study only the body temperatures in the afternoons for the 4-month period of June through September of each year were used. The report covers the five summer periods of 1941 through 1945. All body temperatures used in this report were obtained in the barn, and the cows were grazed on pastures in which shade was available during the test periods of each year.

The heat-tolerance coefficient for each cow was determined by first calculating the body temperature at 90° F. air temperature from linear regres-

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sion equations and then converting this body temperature to a heat-tolerance coefficient by use of the equation $HT = 100 - [14(BT - 101)]$. BT represents the calculated body temperature and HT the heat-tolerance coefficient. This equation is a modification of the one devised by Rhoad (2, 3) and is based on the assumption that a body temperature of 108°+ at 90° F. air temperature and under otherwise normal conditions would indicate complete loss of control in the regulation of body temperature, or zero per cent efficiency in eliminating surplus body heat. Conversely, a body temperature of 101° F. is considered as normal, and a cow that could maintain that body temperature at 90° F. air temperature would be considered 100 per cent efficient in eliminating surplus body heat. The heat-tolerance coefficient is used rather than the body temperature as it is a simpler figure to work with and gives a better expression of the degree of heat tolerance than does the body temperature.

An air temperature of 90° F. is used as a base for determining the body reaction as the maximum air temperature is 90° F. or higher on about 67 per cent of the 122 days in the June-through-September period at this station. Cattle, therefore, must be able to withstand 90° F. temperatures continuously if they are to be considered as well adapted to this area.

Since there is a known regression of body temperature on air temperature, it was considered that calculating the body temperature at 90° F. from regression formulae would be a more accurate method of determining the average body temperature for each animal than using the average body temperature within a specified air temperature range. This method also permits the use of all readings made during the test period and tends to eliminate differences of air temperature from year to year. The number of readings used in calculating the regression coefficient for each cow each year ranged from 10 to 18, with an average of 16.8 readings being used for determining each heat-tolerance coefficient.

One hundred and thirty-seven heat-tolerance coefficients were determined for 74 different cows during the 5-year period. Due to unavoidable circumstances, there was a rapid turnover in the herd during the period under study, and heat-tolerance coefficients were obtained on many of the cows for one year only and on only a few cows for more than 2 years.

EXPERIMENTAL RESULTS

Table 1 lists the number of cows included each year, the average heat-tolerance coefficient for the herd, the highest and lowest heat-tolerance coefficients for an individual in each year, and the range of air temperature encountered during the summer periods. An analysis of variance showed the differences in the yearly means of the heat tolerance to be not significant statistically. (The analyses of variance in this report were made by methods described by Snedecor (7). The term "significant" or "*"

TABLE 1

Heat-tolerance data of all cows, by years

Year	Number of cows	Heat-tolerance coefficients (%)*			Air temperatures (°F.)†		
		Average	Maximum	Minimum	Average	Maximum	Minimum
1941	21	77.8	90	62	86.6	94	78
1942	34	76.1	92	61	86.1	95	77
1943	20	81.2	92	65	88.4	96	80
1944	29	79.2	90	68	90.1	98	79
1945	33	77.6	89	64	85.9	93	77
Total or average	137	78.1	92	61	87.4	98	77

* The maximum is the highest for any individual cow; the minimum, the lowest for any individual cow; the average is that for all the cows.

† The figures given are the average for the four-summer-months periods of June through September and the highest and lowest temperatures encountered while taking the readings in the barn.

means that "*F*" values were between the 5 and 1 per cent points; and the term "highly significant" or "**" means that the "*F*" values were larger than those required at the 1 per cent level.)

Since there seemed to be little difference in the average heat tolerance of the herd from year to year, the data were grouped by age of the cow at the time of determining the coefficient, with the results shown in table 2.

There would seem to be a real difference between the average heat tolerance of the different age groups, since the "*F*" value is greater than that required for significance at the 1 per cent level. Apparently, there is a definite increase in heat tolerance as the cows increase in age from 2 to 3 years and probably some decrease again at ages beyond 8 years. An analysis of variance of the yearly means for each group was made, and the differences were found to be not significant from year to year except for the 3-year-old group. There was a wide variation from year to year in this 3-year-old group, and the "*F*" value for the between-year variance was greater than required for significance at the 1 per cent level. A possible explanation for this difference will be referred to under sire group analyses.

TABLE 2

Heat-tolerance data of all cows, by age groups

Age group*	Number of cows	Heat-tolerance coefficients		
		Average	Maximum	Minimum
6 years and over	27	75.8	92	61
5 years to 5 years 11 months	17	79.0	89	64
4 years to 4 years 11 months	28	79.5	92	65
3 years to 3 years 11 months	38	80.8	92	69
2 years to 2 years 11 months	27	74.7	90	61
Total or average	137	78.1	92	61

* Age of each cow was figured on August 1 of each year (center of test period).

TABLE 3

Correlation of heat-tolerance coefficients

Simple correlation (r) between heat-tolerance coefficients of cows determined at:	r	Number of cows
2 years of age and same cows at 3 years of age	+ 0.24	19
3 years of age and same cows at 4 years of age	− 0.21	21
2 years of age and same cows at 4 years of age	+ 0.47	10
4 years of age and same cows at 5 years of age	+ 0.57*	11
5 years of age and same cows at 6 years of age	+ 0.96**	7
6 years of age or over and next consecutive year	+ 0.84*	5
4 years of age or over and next consecutive year	+ 0.66**	23

* Near to significance as determined from table 7.2 of Snedecor (7).
** Highly significant as determined from table 7.2 of Snedecor (7).

To measure the stability of the heat-tolerance coefficient for the individual cow from year to year, correlations were calculated, with the results shown in table 3.

It is obvious from these results that the heat-tolerance coefficient determined at 2 or 3 years of age would have little value in predicting the heat-tolerance coefficient of an animal at 4 years or more of age. Thus, while there is a definite increase in average heat tolerance from 2 to 3 years of age, as noted above, the increase is not at all uniform among the individuals studied. The heat-tolerance characteristic does appear to be more stable in the older cows. The coefficients of correlation between the heat-tolerance determinations made at 4 and 5 years of age, and between consecutive years at ages above 6 years, approach the 5 per cent level of significance, and the heat-tolerance determinations made at 5 and 6 years of age are almost perfectly correlated. The last group of table 3 is composed of all cows having heat-tolerance coefficients for two consecutive years, made at ages of 4 years or more. The correlation coefficient is higher than that required for statistical significance at the 1 per cent level, indicating that among the older cows there is a reasonable degree of stability in the heat-tolerance characteristic of the individual from one year to the next.

A considerable difference in heat tolerance of individual cows under similar conditions has been regularly noted in the studies here, and an analysis of variance was made to determine if these differences are great enough to be significant. Since the heat tolerance is unstable at the younger

TABLE 4

Analysis of variance of data on body temperatures of individual cows 4 years old and over

Source of variance	*df*	Sum of squares	Mean square
Total	1208	808.49	
Between-individual-cow means	71	242.01	3.409**
Within individual cow	1137	566.48	0.498

** Highly significant.

ages, only those cows were used which had determinations made at 4 years of age or older.

Analyses first were made for each of the five test periods, and the mean square of the between-cow variation was found to be highly significant in every year, the "*F*" values ranging from one and one-half to four times that required for significance at the 1 per cent level. The analysis of variance of the data for 5 years is given in table 4. There would seem to be no question but that there is a real difference in the body response of the individual cows to the climatic factors at this station.

To determine the effect of lactation and gestation on the heat-tolerance coefficient, the data from three groups of cows were studied. Group I cows were milking approximately one-half of a summer period and dry the balance of the same period and were pregnant 180 days or more at the start of the dry period. Group II cows were milking approximately one-half of a summer period and dry the balance of the same period but were pregnant less than 180 days at the start of the dry period. Group III cows were milking all of one summer period and dry all the previous or subsequent summer period. The averages are shown in table 5.

TABLE 5

Comparison of heat tolerance of cows when milking and dry

Group number*	Number of cows	Average heat tolerance		Average days pregnant—start of period	
		Milking	Dry	Milking	Dry
I	18	75.5	77.6	71.8	211.0
II	5	85.8	87.4	61.8	107.2
III	6	76.5	78.8	13.8	100.5

* Group I were in milk approximately half of a summer period and dry the balance of the period and were pregnant 180 days or more at start of the dry period.

Group II were in milk approximately half of a summer period and dry the balance of the period and were pregnant less than 180 days at the start of the dry period. Heat tolerance averages for the milking and dry periods for Groups I and II are, therefore, for the same cows in the same year.

Group III were milking all of one summer period and dry all of the previous or subsequent summer period. Heat tolerance averages for the milking and dry periods are, therefore, for the same cows in different years. Cows that were 4 years old or over in the first year included are the only ones included in this group.

The differences between the average heat tolerance of the cows when milking and when dry are small in all three groups and cannot be considered as significant, since the mean square for within-group variance is larger than that for between milking and dry in each case. Apparently, there is little difference in body reaction regardless of the stage of lactation or gestation.

Since there was a definite difference in the reaction of individual cows, it would be desirable to know if this difference is inherited. A comparison of sire groups is given in tables 6 and 7. Sufficient data are not yet available to attempt to determine the influence of the dam.

TABLE 6

Comparison of heat-tolerance coefficients of groups of daughters of different sires

Sire*	Heat-tolerance coefficients of daughters determined at:					
	2 years of age		3 years of age		4 years or more of age	
	No. of daus.	Average *HT*	No. of daus.	Average *HT*	No. of daus.	Average *HT*
VGM	. .	.	.	. .	4	81.8
571	. .		.		8	79.0
137			5	86.4	4	80.8
715	6	70.2	7	75.3	11	80.0
82	8	78.9	12	81.8	7	78.7
140	6	70.2	10	81.6	5	78.0
60	5	78.4				

* Only those sires having four or more daughters with *HT* determinations are included.

The averages and the analyses of the sire groups are made on heat-tolerance coefficients determined at 2, 3, and 4 years of age and over, separately, since it was shown above that the heat-tolerance coefficients are not stable in the younger cows.

The difference between the mean heat tolerances of the sire groups appears to be fairly definite for the determinations made at 2 and 3 years of age, since the "*F*" values for these two mean squares fall between the 5 and 1 per cent points, but there is apparently no appreciable difference between the sire-group means where the determinations were made at 4 years or more of age. The influence of the sire on the heat tolerance of his daughters would, therefore, appear to be negligible, except at the younger ages.

In the analysis of the yearly means for various age groups (see table 2) it was noted that the means of the 3-year-old group varied significantly from year to year. This is believed due to the fact that in 1941 this group was made up entirely of daughters of 137, with a high average heat tolerance; and in 1942 of daughters of 715, with a low average heat tolerance (see

TABLE 7

Analysis of variance of data on heat-tolerance of sire groups

Group	Source of variance	*df*	Sum of squares	Mean square
I *HT* determined at 2 years of age	Total	24	1,406	.
	Between sire groups	3	454	151.33*
	Within sire groups	21	952	45.33
II *HT* determined at 3 years of age	Total	33	1,299	.
	Between sire groups	3	387	129.00*
	Within sire groups	30	912	30.40
III *HT* determined at 4 years or more of age	Total	38	1,586	. .
	Between sire groups	5	47	9.40
	Within sire groups	33	1,539	46.64

* Significant at 5% level.

table 6). In the other 3 years, the 3-year-old groups were made up of daughters of two or more sires, and the average heat tolerances were not much different from the 5-year average. This is believed to be the only place in this study where the sire influence would bias the results, as all of the other groups (except in tables 6 and 7) are composed of daughters of several different sires.

CONCLUSIONS

The following conclusions would appear to be warranted for Jersey cows under conditions prevailing at this station:

1. Not much change in the average heat tolerance of the herd occurs from year to year.
2. A definite difference in heat tolerance in the different age groups exists with the 2-year-olds showing the lowest average and the 3-year-olds the highest average.
3. The heat-tolerance coefficient is a reasonably stable individual characteristic at ages of 4 years and above, but not at 2 or 3 years of age.
4. There is a real difference in physiological response of different cows to the same environmental conditions, as measured by the body temperature.
5. The stage of lactation and gestation has little, if any, effect on the heat-tolerance coefficient.
6. There is some difference in the response of groups of daughters of various sires when measured at 2 and 3 years of age but little, if any, when measured at 4 years of age or more.

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SYNTHETIC RATIONS FOR THE DAIRY CALF[1]

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Information available up to 1942 on the nutrition of the calf has been adequately reviewed by Savage and McCay (9). Johnson, Loosli, and Maynard (4) used purified diets to study the growth requirements of dairy calves. They reported growth rates which were below normal when compared to Ragsdale's standards (7). They stated that poor food consumption, associated with periodic digestive upsets, seemed to be largely responsible for the slow growth. Madsen, McCay, and Maynard (6) were successful in rearing sheep on synthetic rations for a period of 480 days after weaning, but obtained less satisfactory results with goats, guinea pigs, and rabbits.

In this paper we report the development of a synthetic diet which is satisfactory for the nutrition of the young dairy calf.

EXPERIMENTAL

Male dairy calves 24 to 48 hours old which had been allowed to receive colostrum were used as experimental animals. They were housed in individual metal cages 5 × 6 feet in size and equipped with heavy wire mesh bottoms. When the animals were received, they were given a capsule containing 100,000 I.U. of vitamin A and an injection of anti-scour serum. All diets were compounded to simulate milk. For the first week the calves were fed from pails equipped with rubber nipples, and at the end of this time they were taught to drink from the bucket. The animals were fed at a level of one pound of synthetic milk for each ten pounds of body weight. They were fed twice a day and given half the daily intake at each feeding. All vitamin supplements were given at the morning feeding. The synthetic milk was always fed at a temperature of 37° C.

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The synthetic milk rations contained 13 per cent solids, similar to the solids content of cow's milk. The casein was dissolved according to the method of Bird *et al* (2). Sodium bicarbonate equal to 4.75 per cent of the weight of the casein was added to water at a temperature of 60° C. The casein was added slowly to the sodium bicarbonate solution and stirred with constant high speed (a "Lightnin" mixer was used[3]). When the casein was in solution, cerelose was added. The salt mixture was dissolved in boiling water and then added to the casein-cerelose solution. After the entire mixture had been stirred for approximately one hour, the fat was homogenized into the mixture under a pressure of approximately 3,000 lbs. The synthetic milk was then pasteurized and stored in a refrigerator at a temperature of 5° C. until used. The synthetic milk thus prepared had a

TABLE 1

Composition of synthetic milks

Components	Ration 1	Ration 2	Ration 3	Vitamins added[5]	Mg. per kg. liquid milk Ration 1	Mg. per kg. liquid milk Ration 2	Mg. per kg. liquid milk Ration 3
	%	%	%				
Casein "Labco"	30.0	30.0	30.0	Thiamin	0.39	0.65	0.65
Soybean oil	12.0			Riboflavin	1.95	0.65	0.65
Lard		26.6	26.3	Pyridoxine	0.65	0.65	0.65
Salts 1[1]	6.0	6.0		Calcium pantothenate	1.95	1.30	1.30
Salts 2[2]			4.0				
Cerelose	47.0	37.4	39.4	Nicotinic acid		2.60	2.60
Wheat germ oil			0.3[4]	Ascorbic acid		13.0	
Liver[3]	5.0			α-Tocopherol		1.0[6]	
				2-Methyl-1,4-naphthoquinone		0.26[6]	0.26[6]
				Inositol			26.0
				Choline			260.0
				p-Aminobenzoic acid			2.60
				Pteroyl-glutamic acid (folic acid)			0.052
				Biotin			0.01
				Vitamin A	5,000 I.U. per day		
				Vitamin D	500 I.U. per day		

[1] Salts 446 used in this laboratory, unpublished.

[2] Salt mixture of Phillips, P. H., and Hart, E. B., Jour. Biol. Chem., 109: 657 (1935), and modified by addition of cobalt chloride and increasing the manganese content. These salts were preferred because they dissolved more readily in the liquid rations.

[3] A defatted pork liver prepared and donated by the VioBin Corporation, Monticello, Illinois, through the courtesy of Mr. Ezra Levin.

[4] The 2-methyl-1,4-naphthoquinone, dissolved in the wheat germ oil, and the lard were homogenized into the solution of casein, salts, and cerelose.

[5] The water-soluble vitamins were made up in 25 per cent alcohol.

[6] The α-tocopherol and 2-methyl-1,4-naphthoquinone were dissolved in 95 per cent alcohol.

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[3] Manufactured by Mixing Equipment Co., Rochester, New York.

pH of 6.5 to 6.8. The compositions of the liquid rations used are given in table 1.

A capsule containing 5,000 I.U. vitamin A, 500 I.U. vitamin D, 50 mg. nicotinic acid, and 250 mg. ascorbic acid was given each animal daily.

Two animals, placed on ration 1, appeared to be doing well at the end of one week. During the second week both animals began to scour and appeared to be excreting a considerable amount of fatty material. The scours became progressively worse, and the animals refused to eat, lost weight, and presented a very unthrifty appearance. An attempt was made to save the animals by substituting a diet of cow's milk and treating scours by administration of sulfathalidine. Calf no. 1 became worse and died. Post-mortem examination showed the cause of death to be infectious scours. The condition of calf no. 2 improved on the diet of cow's milk and the animal finally recovered.

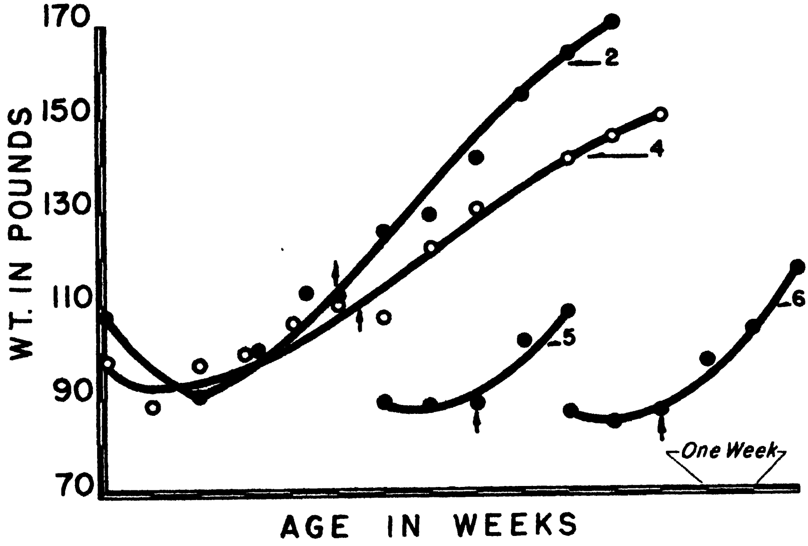

FIG. 1. Growth of calves on soybean oil–skim milk and lard–skim milk rations. Upward-pointing arrow indicates change from soybean oil–skim milk ration to lard–skim milk ration.

Gullickson, Fountaine, and Fitch (3) have reported that calves fed a diet of soybean oil homogenized into skim milk grew poorly, scoured, and appeared emaciated and unthrifty. Since soybean oil was included in ration 1, an attempt was made to determine whether this oil could be used satisfactorily in calf rations. Calves nos. 2, 3, 4, 5, and 6 were placed on a diet of skim milk into which soybean oil was homogenized at a level of 4 per cent on the liquid basis. After several days on this diet the animals started to scour, became very unthrifty in appearance and grew poorly. The volume of fecal material was large and appeared to contain large quantities of undigested matter. The feces contained up to 30 per cent ether extract on the dry basis.

On the soybean oil–skim milk diet, calf no. 3 had severe scours, lost weight and became very weak. The animal, changed to a diet of whole cow's milk, still did not improve and was taken off the experiment. Autopsy showed the animal had gastro-enteritis.

Since the other animals (calves nos. 2, 4, 5, 6) were not doing well on this diet, it was decided to try some other fat. Lard was selected because the work of Gullickson *et al.* (3) had shown that calves fed a diet of lard homogenized into skim milk made good gains in weight, remained in a healthy and thrifty condition, and exhibited only occasional scours. The animals were changed to a diet of skim milk into which lard was homogenized at a level of 4 per cent on the liquid basis. After several days on this diet the animals stopped scouring, became healthy and thrifty in appearance, and made gains in weight. The growth data are summarized in figure 1.

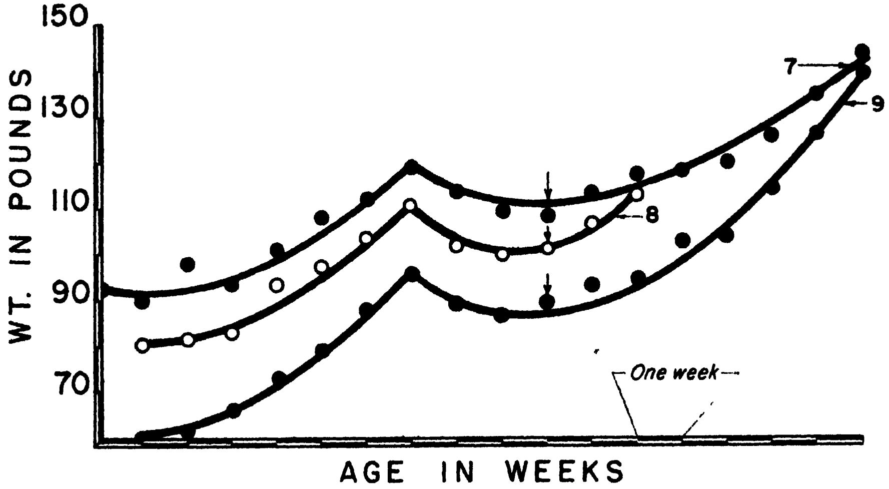

FIG. 2. Growth of calves on rations 2, 4, and 5. Break in curve at 7 weeks occurred with change to ration 4. Downward-pointing arrow indicates change to ration 5.

From these results it was apparent that the type of fat used in the diet of the dairy calf is of importance. Ration 2 was prepared and homogenized with water, as previously described.

Three animals, calves nos. 7, 8, and 9, were placed on ration 2. They made good gains in weight, appeared healthy and thrifty, and scoured infrequently. The growth data are given in figure 2.

After 6 weeks on the liquid diet, a change to a solid diet was attempted. The animals would not eat the dry ration except when fed as a slurry. The composition of ration 4 is given in table 2. On this ration the animals scoured and lost weight. Decreasing the lard content to 4 per cent, changing the salt mixture, or adding roughage (wood flock) did not prevent scours. Even the addition of *p*-aminobenzoic acid, inositol, choline, pteroylglutamic acid ("folic acid") and biotin did not improve the condition of the animals. Only after the substitution of starch for some of the cerelose did the animals stop scouring and gain weight. The composition of ration 5, on which the calves showed good growth, appeared healthy and thrifty, and did not scour, is given in table 2.

Rations 1 and 2, previously described, did not give completely satisfactory results for rearing young calves. The vitamin supplement was modified to contain all the pure crystalline compounds that have been reported to be members of the vitamin B complex. Two animals have been raised on ration 3 for a period of 12 weeks. The growth of the calves was normal as compared to Ragsdale's standards (7), and they were healthy and thrifty in appearance. The scours occurring on two or three occasions were cured by administration of sulfathalidine. The growth data are given in figure 3.

TABLE 2

Composition of dry rations

Components	Ration 4	Ration 5	Vitamins added	Mg. per kg.	
				Ration 4	Ration 5
	%	%			
Casein "Labco"	20.0	20.0	Thiamin	5.0	5.0
Lard .	8.0	4.0	Riboflavin	5.0	5.0
Salts 1	6.0		Nicotinic acid	20.0	20.0
Salts 2		4.0	Pyridoxine	5.0	5.0
Magnesium carbonate	0.7		Calcium pantothenate	10.0	10.0
Cerelose	65.3	20.0	Ascorbic acid	100.0	
Methionine		0.3	*p*-Aminobenzoic acid		20.0
Wheat germ oil		0.3	Inositol		200.0
Wood flock		10.0	Choline		2,000.0
Starch		41.4	Pteroyl-glutamic acid (folic acid)	.. .	0.4
			Biotin		0.1
			2-Methyl-1,4-naphthoquinone		2.0
			Vitamin A	5,000 I.U. per day	
			Vitamin D	500 I.U. per day	

Ascorbic acid was not included in the vitamin supplement of ration 3 as it was found unnecessary for the young calf. Several calves have been raised without vitamin C in the diet and showed normal growth, appeared healthy and thrifty, and did not scour. In addition, they did not develop navel ill, which Lundquist and Phillips (5) reported to be caused by a vitamin C deficiency. Blood ascorbic acid levels have been determined by the method of Roe and Keuther (8) and are given in table 3. The values are normal by comparison to those reported by Lundquist and Phillips (5), who found the ascorbic acid content of calf blood varied between 0.1 and 0.8 mg. per 100 cc. of whole blood.

TABLE 3

Ascorbic acid levels of calves' whole blood

Calves on diet containing ascorbic acid						Calves on ascorbic acid–free diet											
Calf 7		Calf 8		Calf 9		Calf 10		Calf 11		Calf 12		Calf 13		Calf 14		Calf 15	
Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.	Age in days	Mg./ 100 cc.
45	0.25	30	0.42	30	0.82	7	0.75	2	0.81	2	0.60	2	0.94	2	1.04	2	0.72
52	0.50	37	0.94	37	0.66	14	0.77	9	0.29	9	0.47	8	0.80	8	0.63		
59	0.51	44	0.83	44	0.70	21	0.90	16	0.53	16	0.55	23	0.39	23	0.38		
73	0.46			79	0.59	28	0.93	23	0.58	23	0.76						
								30	0.77	30	0.47						
								37	0.49	37	0.51						
								44	0.66	44	0.84						
										51	0.57						

Ascorbic acid was fed to calf no. 7 until 70 days of age and to calves nos. 8 and 9 until 56 days of age. After this time these animals received an ascorbic acid–free diet. Calves nos. 10, 11, 12, 13, 14, and 15 did not receive any ascorbic acid during the course of the experiment. The data in table 3 show that the blood level of ascorbic acid does not decrease to any great extent when the animal is fed an ascorbic acid–free diet. This indicates that the calf can synthesize vitamin C in its body tissues and does not need it in the diet.

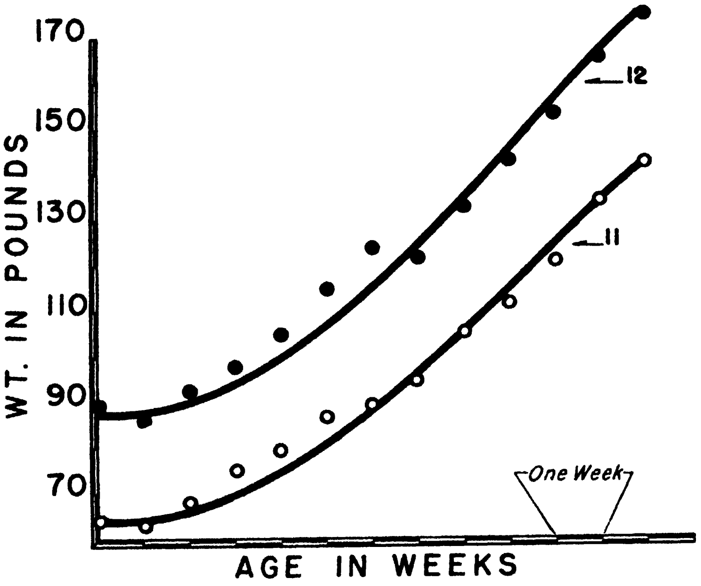

FIG. 3. Growth of calves on ration 3.

DISCUSSION

A synthetic milk diet (ration 3) has been developed for studies of the nutrition of the young dairy calf. Day-old calves have been raised on this ration for a period of 12 weeks. They have shown good growth, were healthy and thrifty in appearance, and seldom scoured.

When the ration was fed in the dry form, less satisfactory results were obtained. It may be that the physical characteristics of this diet or the method of feeding (as a slurry) caused the unsatisfactory results. Bate, Espe, and Cannon (1) have reported that dairy calves did poorly on a diet of skim milk and unhomogenized fat. Calves fed the same diet but with the fat homogenized into the skim milk did better. Since rations 4 and 5 were fed as slurries, the fat was unhomogenized. This may have been the cause of the poor results obtained.

The data presented in table 3 on the blood levels of ascorbic acid indicate that the young dairy calf does not require ascorbic acid. Lundquist and Phillips (5) have reported that the young dairy calf requires ascorbic acid only if the animal is receiving an insufficient amount of vitamin A. The animals in the experiments reported in this paper all received 5,000 I.U. of vitamin A per day. This may account for the fact that the results obtained do not show a great decrease in the blood level of ascorbic acid.

SUMMARY

1. A synthetic milk has been developed for the young dairy calf. This ration will support good growth over a period of 12 weeks from birth. The animals raised on this diet were healthy and thrifty in appearance.

2. On rations containing liberal amounts of vitamin A, the young dairy calf does not require vitamin C.

3. Calves that received soybean oil in the diet grew poorly, scoured, and appeared unthrifty, whereas the animals that received the rations prepared with lard showed good gains in weight, did not scour, and appeared normal and healthy.

4. The calves did much better in all respects when fed the synthetic milk diet than when solid diets fed as a slurry were used.

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A SUGGESTED MODIFIED BABCOCK PROCEDURE FOR TESTING HOMOGENIZED MILK[1]

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With increasing consumer acceptance of homogenized milk, considerable interest has been shown in the testing of such milk for fat either by the standard Babcock method or by a modification. In general, early work and statements conflicted relative to the accuracy of the Babcock test for homogenized milk. However, there was general agreement that the homogenized milk tested within the 0.1 per cent tolerance of the Babcock procedure. Some accepted the theory that the fat globules, being reduced in size by the process, could not be centrifuged sufficiently to give a test comparable to that of nonhomogenized milk; consequently, the Babcock fat test of homogenized milk could be expected to be not more than 0.1 per cent lower than that of nonhomogenized milk.

If a slightly lower test value were the only factor involved in making a Babcock fat test of homogenized milk, likely the regular procedure would be accepted and used in routine work. However, workers soon recognized that char formation was greater in applying the test to homogenized than to nonhomogenized milk. This char often gave the appearance of a burned test. If less acid were used, the test often appeared curdy. This char or curd usually appeared as a thin disc or plug at the base of the fat column. When the char was reduced to a minimum, its presence did not affect appreciably the reading of the fat column. In routine testing, however, the presence and appearance of this char in greater or lesser amounts, intermixed with or at the base of the fat column, often led one to question the accuracy of the test. Consequently, many modifications of the Babcock procedure, in part with the aim of preventing char formation and thus increasing the accuracy of the test, have been recommended for testing homogenized milk.

The literature on this subject has been reviewed, in part, in a previous paper (9), which showed that at least 12 modifications of the standard Babcock procedure have been recommended for homogenized milk. Variants involved in the different modifications included: (a) strength of the sulphuric acid used, (b) amount of acid used, (c) temperature of the acid, (d) addition of acid ranging from three to five portions, (e) temperature of the milk, (f) prolonged mixing of acid and milk, (g) remixing acid-water-serum mixture after centrifuging, and (h) prolonged centrifuging.

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EXPERIMENTAL METHODS

In comparing the various modifications recommended, it became evident that the procedures involving prolonged action of the sulphuric acid at a relatively high concentration without burning the test offered the best solution to the problem. This, in effect, entailed the tempering of the milk and acid to 70° F., the use of a relatively large portion of the acid (1.83 sp. gr. sulphuric acid) at the first addition, thorough mixing of the acid and milk after each addition, and prolonged agitation of the milk-acid mixture after all the acid was added. As a result of these observations and after comparing the numerous modifications of the test, the following suggested modified Babcock procedure for testing homogenized milk was adopted:

(a) Temper the acid and milk to 70° F.
(b) Use sulphuric acid of 1.83 to 1.835 sp. gr.
(c) Use the full amount of sulphuric acid (17.5 ml.).
(d) Add the acid in three portions, 8, 5, and 4.5 ml., respectively.
(e) Mix the acid and milk by rotary motion after each addition and continue agitation for at least 15 seconds before adding the second and third increments of sulphuric acid.
(f) Shake the tests in a mechanical shaker for at least 2 minutes before centrifuging.
(g) Centrifuge and add hot water in accordance with the regular Babcock procedure. Substitution of a water-alcohol (ratio 1.4:1 by weight) solution for the hot water to bring the fat up into the neck of the bottle for reading is optional.

This procedure was followed carefully in testing in duplicate a series of 36 milk samples, both nonhomogenized and homogenized. These samples also were tested in duplicate by the Mojonnier method, which was used as a standard for accuracy. However, instead of using a volumetrically measured approximately 10-gram portion, the samples previously tempered to 70° F. were weighed carefully on a chemical balance directly into fat-extraction flasks.

The nonhomogenized samples were taken from the vat after pasteurization prior to homogenization. The milk had been kept thoroughly mixed during pasteurization and homogenization in order to insure uniform fat distribution. The homogenized samples were taken from the cooled, bottled product after homogenization was well under way.

Homogenization was accomplished by means of a 500-gallon-per-hour viscolizer at 2500 pounds pressure at 130° F. following pasteurization. All the 17.5-ml. portions of milk were pipetted into the calibrated test bottles at one time to assure correct sampling after the milk had been tempered at 70° F. for two hours.

In studying the accuracy of the suggested modified Babcock procedure, the procedure was tried on the same milk unhomogenized and homogenized.

TABLE 1

Percentages of fat in nonhomogenized and homogenized milk as determined by the Mojonnier method

Trial no.	Date	Mojonnier fat test on milk which was						Variation of average fat test of homogenized milk from that of nonhomog. milk
		Not homogenized			Homogenized			
		Duplicates	Difference between duplicates	Average test	Duplicates	Difference between duplicates	Average test	
		%		%	%		%	
1	12–13–44	3.735 3.698	0.037	3.713	3.705 3.722	0.017	3.714	+ 0.001
2	12–14–44	3.928 3.933	0.005	3.930	3.962 3.943	0.019	3.952	+ 0.022
3	12–15–44	4.024 4.047	0.023	4.035	4.058 4.073	0.015	4.064	+ 0.029
4	12–18–44	3.973 3.957	0.016	3.965	3.976 3.976	0.000	3.976	+ 0.011
5	12–19–44	3.918 3.867	0.051	3.892	3.888 3.903	0.015	3.895	+ 0.003
6	12–20–44	3.984 4.033	0.049	4.008	4.064		4.064	+ 0.056
7	12–21–44	4.068		4.068	3.994 3.948	0.046	3.971	– 0.097
8	1–15–45	3.966 3.906	0.060	3.936	3.931 3.888	0.043	3.910	– 0.026
9	1–16–45	3.702		3.702	3.706		3.706	+ 0.004
10	1–17–45	3.898 3.899	0.001	3.898	3.876 3.887	0.011	3.881	– 0.017
11	1–18–45	3.848 3.849	0.005	3.848	3.847 3.868	0.021	3.857	+ 0.009
12	1–19–45	3.980 3.973	0.007	3.977	3.982 3.996	0.014	3.989	+ 0.012
13	1–22–45	5.012 5.038	0.026	5.025	5.049 5.040	0.009	5.044	+ 0.019
14	1–23–45	4.956 4.973	0.017	4.964	4.936 4.934	0.002	4.935	– 0.029
15	1–24–45	4.575 4.628	0.053	4.601	4.653 4.634	0.019	4.643	+ 0.042
16	1–25–45	5.124 5.082	0.042	5.103	5.047 5.082	0.035	5.064	– 0.039
17	1–26–45	4.470 4.476	0.006	4.473	4.493 4.503	0.010	4.498	+ 0.025
18	1–29–45	4.591 4.589	0.002	4.590	4.580 4.600	0.020	4.590	0.000
19	1–30–45	4.763 4.761	0.002	4.762	4.759 4.768	0.009	4.763	+ 0.001
20	1–31–45	4.619 4.606	0.013	4.612	4.608 4.606	0.002	4.607	– 0.005
21	2– 1–45	4.532 4.537	0.005	4.534	4.543 4.552	0.009	4.547	+ 0.013
22	2– 2–45	4.585 4.575	0.010	4.580	4.560 4.570	0.010	4.565	– 0.015
23	2– 5–45	4.375 4.409	0.034	4.392	4.407 4.404	0.003	4.405	+ 0.013
24	2– 6–45	4.606 4.627	0.021	4.616	4.641 4.644	0.003	4.642	+ 0.026
25	2– 7–45	4.591 4.596	0.005	4.593	4.597 4.586	0.011	4.591	– 0.002
26	2– 8–45	4.786 4.789	0.003	4.789	4.785 4.777	0.008	4.781	– 0.006
27	2– 9–45	4.556 4.592	0.036	4.574	4.594 4.593	0.001	4.593	+ 0.019
28	2–19–45	3.831 3.804	0.027	3.818	3.804 3.815	0.011	3.810	– 0.008
29	2–20–45	3.708 3.699	0.009	3.704	3.701 3.694	0.007	3.698	– 0.006
30	2–22–45	3.651 3.650	0.001	3.650	3.672 3.671	0.001	3.671	+ 0.021
31	2–23–45	3.897 3.926	0.029	3.912	3.918 3.903	0.015	3.911	– 0.001
32	2–26–45	3.723 3.716	0.007	3.720	3.724 3.738	0.014	3.731	+ 0.011
33	2–27–45	3.695 3.716	0.021	3.706	3.712 3.695	0.017	3.704	– 0.002
34	2–28–45	3.850 3.864	0.014	3.857	3.835 3.832	0.003	3.834	– 0.023
35	3– 1–45	3.814 3.801	0.013	3.807	3.826 3.817	0.009	3.821	+ 0.014
36	3– 2–45	3.916 3.886	0.030	3.901	3.898 3.882	0.016	3.890	– 0.011
Averages		(Arithmetic) (Algebraic)	0.0189	4.2015		0.0124	4.2032	0.0177 + 0.0017

Thus, comparisons are made on homogenized milk tested under the same conditions as the nonhomogenized samples. In other words, the regular Babcock procedure was not used as a standard for the nonhomogenized milk. It was believed that no modification of the Babcock procedure would improve the accuracy of the regular Babcock procedure on nonhomogenized

TABLE 2

Comparison of modified Babcock and Mojonnier tests on homogenized milk

Trial no.	Date	Modified Babcock test of milk			Variation of modified Babcock test from Mojonnier test	
		Not homogenized Average of duplicates	Homogenized Average of duplicates	Difference	Not homo genized	Homogenized
		%	%			
1	12–13–44	3.725	3.70	-0.025	+0.008	-0.014
2	12–14–44	4.00	4.00	0.00	+0.070	+0.048
3	12–15–44	4.10	4.10	0.00	+0.065	+0.036
4	12–18–44	4.10	4.10	0.00	+0.135	+0.124
5	12–19–44	3.95	3.95	0.00	+0.058	+0.055
6	12–20–44	4.10	4.05	-0.05	+0.092	+0.014
7	12–21–44	4.05	4.025	-0.025	-0.018	+0.054
8	1–15–45	4.00	4.00	0.00	+0.064	+0.090
9	1–16–45	3.80	3.775	-0.025	+0.098	+0.069
10	1–17–45	3.925	3.90	-0.025	+0.027	+0.019
11	1–18–45	3.90	3.90	0.00	+0.052	+0.043
12	1–19–45	4.025	4.00	-0.025	+0.048	+0.011
13	1–22–45	5.075	5.125	+0.05	+0.050	+0.081
14	1–23–45	5.00	4.95	-0.05	+0.036	+0.015
15	1–24–45	4.675	4.65	-0.025	+0.074	+0.013
16	1–25–45	5.175	5.15	-0.025	+0.072	+0.086
17	1–26–45	4.525	4.525	0.00	+0.052	+0.027
18	1–29–45	4.65	4.625	-0.025	+0.060	+0.035
19	1–30–45	4.80	4.80	0.00	+0.038	+0.037
20	1–31–45	4.70	4.70	0.00	+0.088	+0.093
21	2– 1–45	4.575	4.575	0.00	+0.041	+0.028
22	2– 2–45	4.625	4.625	0.00	+0.045	+0.060
23	2– 5–45	4.475	4.475	0.00	+0.033	+0.070
24	2– 6–45	4.675	4.70	+0.025	+0.059	+0.058
25	2– 7–45	4.625	4.625	0.00	+0.032	+0.034
26	2– 8–45	4.80	4.80	0.00	+0.013	+0.019
27	2– 9–45	4.675	4.60	-0.075	+0.101	+0.007
28	2–19–45	3.85	3.85	0.00	+0.032	+0.040
29	2–20–45	3.725	3.70	-0.025	+0.021	+0.002
30	2–22–45	3.725	3.70	-0.025	+0.075	+0.089
31	2–23–45	3.95	3.925	-0.025	+0.038	+0.014
32	2–26–45	3.775	3.775	0.00	+0.055	+0.044
33	2–27–45	3.775	3.75	-0.025	+0.069	+0.046
34	2–28–45	3.90	3.875	-0.025	+0.043	+0.041
35	3– 1–45	3.90	3.875	-0.025	+0.093	+0.054
36	3– 2–45	3.975	3.925	-0.05	+0.074	+0.035
Averages	(Arithmetic)	4.258	4.244	0.018	0.058	0.045
	(Algebraic)			-0.014	+0.057	+0.044

milk. Consequently, the modified Babcock technique was used on the nonhomogenized milk also, and the results accepted as though they were obtained by the regular procedure. A few trials run on nonhomogenized milk by the regular and modified procedures showed the tests to be comparable in every respect.

RESULTS

The data are presented in tables 1 and 2. The average tests of the 36 nonhomogenized and homogenized samples, as determined by the Mojonnier method, were practically identical; the homogenized milk averaged 0.0017 per cent higher than the same milk not homogenized. A study of the duplicate Mojonnier tests showed less variation, on the average, between the tests of homogenized milk than between those of the nonhomogenized milk, although there were several exceptions.

The modified Babcock method yielded tests on homogenized milk on the average within 0.018 per cent of that of the same milk not homogenized. Of the 36 tests, 16 (or 44 per cent) checked with those of the nonhomogenized milk; 15 (or 41 per cent) were 0.025 per cent lower; one was 0.025 per cent higher; two were 0.05 per cent lower; one was 0.05 per cent higher; and one was 0.075 per cent lower. The average algebraic difference was −0.014 per cent lower than that of the nonhomogenized milk.

DISCUSSION

In presenting the suggested modified Babcock procedure for testing homogenized milk, no claim is made for originality. The suggested technique resulted from observations made while testing homogenized milk by the many recommended procedures, results of which already have been published (9). Particular use was made of the principles involved in some of the methods (1, 5, 6, 7, 8, 10). During this earlier study (9) it appeared necessary to prolong action of the sulphur acid on the milk at a concentration to give maximum digestion without burning. Also, continued agitation of the acid-milk mixture following final addition of the acid seemed to facilitate more complete digestion of the caseous matter. These modifications, while involving more time than the regular Babcock method, did not require as much time as did some of the suggested methods. Considerable importance is associated with the correct temperature, both of the acid and of the milk. The procedure used was based on temperatures of 70° F. With this temperature a relatively large volume of sulphuric acid could be introduced into the milk at the first addition without harmful results. Maintaining maximum chemical reaction through further additions of acid and prolonged agitation before centrifuging seem imperative.

Also, importance is associated with the addition of the full volume (17.5 ml.) of sulphuric acid rather than reducing the total volume. This seems to be in accordance with the work of Bailey (3), who showed in his extensive studies on the Babcock test that casein was digested with more difficulty when fat was homogenized into the solution. Data indicated that, as the volume of acid was increased, a lesser percentage of insoluble organic material remained in the mixture.

The feature of remixing the acid-milk mixture after either the first or second centrifugings, and before or after the addition of all or a part of the water (1, 5, 6, 7, 10), was not incorporated into the suggested technique. Such remixing would seem to indicate that the caseous matter was not wholly digested at the time of centrifuging. Apparently if the original mixing and agitation under the best conditions of temperature and concentration of acid were insufficient to digest all the adsorbed casein, then lower temperatures, which naturally follow during the carrying out of the procedure, and a diluted acid, due to the addition of water, would not facilitate further digestion. Furthermore, by remixing the centrifuged fat with the acid-serum-water-liquid, a partial loss of the benefits of the preceding centrifuging would seem to occur. Retention of the caseous matter in its digested form through the addition of hot water (140° F. or above) or other liquids (2, 3) in bringing the liberated fat up into the neck of the bottle for reading seems important. Beautifully clear fat columns were obtained when a hot sulphuric acid–water mixture (7:10) was added instead of water after the centrifugings to bring the fat up for reading (2). However, one lot of 12 tests made using this variant averaged 0.18 per cent lower on the homogenized milk than on the nonhomogenized milk; in another series of 15 samples, the difference was 0.12 per cent lower. Hence, the introduction of an acid-water mixture instead of hot water to raise the fat offered no possibilities.

Brueckner's (4) recommendation of adding a water-alcohol mixture (ratio 1.4:1 by weight) to the tests of homogenized milk to bring the fat up into the column before final centrifuging appears to have considerable merit. Despite any char formation, the tendency of which seems to be reduced, the fat column is clear, is supported by a clear solution, and has well-defined menisci. However, the results of a few trials indicate that the fat-test reading is slightly higher when the water-alcohol solution is used to support the fat column of homogenized milk than when water alone was used. This slightly increased reading may offset, in part, the slightly lower test generally reported on Babcock tests of homogenized milk. Several factors, such as the slight solubility of alcohol in fat and *vice versa,* may contribute to the slightly higher reading of the fat column of homogenized milk when using the water-alcohol mixture to force up the fat, but the appearance of the fat column with its relatively deep menisci supported by a perfectly clear liquid would indicate that such fat columns were in the best condition for reading. The menisci were well defined, probably due to the drying and cleaning action of the alcohol on the glass, thereby enhancing the capillarity of the fat. Thus, the seemingly increased depth of menisci might be sufficient to account for the slightly higher reading. Reports of use of the water-alcohol solution to bring up the fat column on Babcock tests of homogenized milk in routine testing in a commercial laboratory indicate much satisfaction over its use.

In view of the many modifications of the Babcock method for testing homogenized milk, the authors are extremely reluctant to suggest another technique. However, in the interest of clarifying and unifying the procedure with a minimum of time involved, the apparently most satisfactory features of the various methods were incorporated into one method. It is hoped that this procedure will simplify and lend greater accuracy to the Babcock testing of homogenized milk, rather than add to the confusion.

SUMMARY

A modified Babcock fat test for homogenized milk, employing the best features of the many modifications now recommended, has been suggested. The technique involves tempering the milk and acid to 70° F.; using at least 17.5 ml. of sulphuric acid with sp. gr. of 1.83 to 1.835; adding the acid in three portions, the first consisting of approximately one half of the acid; and prolonging the agitation of the milk-acid mixture prior to centrifuging. The addition of a water-alcohol mixture (ratio 1.4 : 1 by weight) to the test instead of water alone before final centrifuging adds to the clarity of the fat column without appreciably affecting the reading.

Employing the suggested technique, the arithmetic-average fat test of 36 samples of homogenized milk was 0.018 per cent lower than that of the same test applied to similar milk not homogenized. The arithmetic-average variations of the 36 tests from the Mojonnier tests were + 0.058 and + 0.045 per cent for nonhomogenized and homogenized milk, respectively.

Credit is due Mr. Robert Frantz for the making of the tests reported herein.

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METHODS OF PRESERVING GRASS SILAGE AND VITAMIN A POTENCY OF MILK PRODUCED THEREFROM[1]

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In previous publications a number of methods for the preservation of grass forages have been reported (3, 7). The investigation has been continued with several forages to which were added various materials to aid in preservation. In addition to chemical analysis of the forages and silages, short-time feeding tests have been made on all the silages to determine their palatability, and longer feeding trials have been made on the most important lots in order to obtain data regarding milk production and the vitamin A potency of the milk produced by cows fed these silages. The work has been extended to several farms which were part of an experimental grass silage program sponsored by the College of Agriculture.

EXPERIMENTAL

Silages. In preliminary experiments, forages treated in different ways were ensiled in layers in silos 8 or 10 feet in diameter. Each layer contained one to 5 tons of forage. The preservative was added to the forage as it was passed through the silage cutter in order to obtain uniform distribution. When the silo was opened, the silages were analyzed and fed, and the readiness with which the cows consumed each lot was recorded. The methods of preservation which had been most successful in the layer trials were further tested on a larger scale. Quantities of fresh forage ranging from 5 to 20 tons were ensiled and the resulting silages were used for feeding experiments. Chemical analyses of the forage and silage were made. Dry matter was obtained by drying in an electric oven at 105° C., and the pH of the silage was determined on the expressed juice by means of a glass electrode. The carotene content was determined by the method of Hegsted, Porter, and Peterson (2), except that a photoelectric colorimeter and a calibration curve of β-carotene in Skelly solve were used instead of a spectrophotometer. Light absorption by the carotene solution was measured with a 440-μ filter.

Milks. The silages used in the feeding trials were fed to lots of from five to nine cows each. The animals composing each lot were selected so that lactation, weight, and milk production and butterfat production were approximately equal at the time the feeding trials were begun. Representative samples of morning and evening milkings were obtained from each lot of animals and

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analyzed for their butterfat, carotene, and vitamin A content. The carotene and vitamin A contents of these milks were determined by the methods of Olson, Hegsted, and Peterson (4) and Berl and Peterson (1). The method used for the extraction and saponification of the butterfat in the milk was that of Olson *et al.* The procedures used for drying the ethereal extract, analysis for β-carotene in Skelly solve and vitamin A in chloroform were those of Berl and Peterson (1).

RESULTS

Layer silages. Table 1 summarizes the data for the layer silage experiments. In silo I, 9 different materials or treatments were tested to determine their usefulness as a means of preservation. Acidity, which is usually a good index of quality, ranged from pH 4.2 to 5.2. The best layers from the standpoint of palatability were those approximating 4.5 or less. Silages which have a pH above 4.5 often contain butyric acid, ammonia, and other fermentation products having an objectionable odor. The layers of alfalfa preserved with Silogerm (a commercial bacterial culture for the preservation of silage) and salt (I–2), and salt alone (I–4) had undergone a butyric type of fermentation and were of poor quality. Carotene preservation in these two silages was reasonably good, however. A butyric fermentation and carotene preservation not uncommonly go together. Butyric fermentation occurs under anaerobic conditions, and exclusion of air favors carotene preservation. The wilted alfalfa and the alfalfa ensiled without a preservative were well preserved and palatable, showing that at times good silage may be obtained without any additions. However, these layers were far down in the silo, where there was considerable pressure from above, and this condition may have favored preservation. The most palatable silages were produced by addition of corn meal, molasses, or concentrated soured whey to the forage. Concentrated soured whey was produced from whey which had been inoculated with a 1 per cent inoculum of *Lactobacillus bulgaricus* and incubated under anaerobic conditions at 110° F. for 5 days. The soured whey was concentrated in vacuo to one-seventh of the original volume and applied to the forage at a rate of 70 pounds per ton. The layers preserved with concentrated soured whey and whey alone were very soggy. There was no correlation between carotene and pH or between carotene and palatability.

The differences between the dry-matter content of the forage and the corresponding silage noted in tables 1 and 2 may be due, in part, to variations in sampling, but in some cases are due to additions either of liquid (*e.g.*, whey) or of dry material (*e.g.*, dry sorghum fodder) to the forage at the time of ensiling. There also would be a tendency for the moisture content of the layers to become equal because of the movement of liquid or water vapor from one layer to the next.

In 1941, the unavailability of molasses led to further experiments (layers II–1 to II–9) in search of a suitable method for preserving grass silage by

TABLE 1

Composition of layer silages

Silo and layer[a]	Year	Forage	Addition or treatment	Preservative	Dry matter		pH	Carotene (dry basis)		Palatability[c]
					Fresh forage	Silage		Fresh forage	Silage	
				lbs./ton	%	%		*μg./gm.*	*μg./gm.*	
I–1	1940	Clover-timothy	Corn meal	150	36.2	36.5	4.3	130	86	Good
I–2	1940	Alfalfa	Silogerm and salt	[b]	28.1	22.1	5.2	192	118	Poor
I–3	1940	Alfalfa	Dry sorghum fodder	250	27.3	26.0	4.5	164	65	Good
I–4	1940	Alfalfa	Salt	10	27.3	23.5	5.2	164	142	Poor
I–5	1940	Alfalfa	None	None	27.3	25.4	4.6	164	83	Good
I–6	1940	Alfalfa	Wilted, 37% D.M.	None	48.6	35.3	4.8	92	70	Good
I–7	1940	Alfalfa	Wilted, 37% D.M., molasses	60	37.8	35.9	4.3	145	81	Excellent
I–8	1940	Alfalfa	Conc. soured whey	70	26.9	25.0	4.3	146	73	Good
I–9	1940	Alfalfa	Wilted, whey	600	42.2	32.5	4.2	100	80	Fair
II–1	1941	Sweet clover (3)–corn (1)	None	None	29.3	30.5	4.1	91	86	Good
II–2	1941	Sweet clover (1)–corn (1)	None	None	28.0	24.5	3.8	83	70	Good
II–3	1941	Alfalfa	None	None	22.9	22.8	4.7	172	5	Poor
II–4	1941	Alfalfa	Silogerm and salt	[b]	22.9	23.0	5.2	172	34	Poor
II–5	1941	Alfalfa	Vacatone	30	21.9	22.1	5.1	172	28	Good
II–6	1941	Alfalfa	Vacatone	60	21.9	24.6	5.0	172	47	Good
II–7	1941	Alfalfa	Wilted, 40% D.M.	None	43.3	25.6	4.9	72	87	Fair
II–8	1941	Alfalfa	Ground barley	200	31.8	31.7	4.4	172	117	Fair
II–9	1941	Alfalfa	Corn and cob meal	250	31.8	30.2	4.0	172	151	Excellent
III–1	1942	Alfalfa	Ground shelled corn	200	41.0	31.9	4.8	75	31	Good
III–2	1942	Alfalfa	Whole shelled corn	200	41.0	30.2	4.5	75	46	Good
III–3	1942	Alfalfa	Ground wood shavings	200		33.7	4.6		39	Poor
III–4	1942	Alfalfa	Oat straw	200	34.5	38.0	4.7	133	30	Poor
III–5	1942	Alfalfa	Dry sorghum fodder	200	29.9	43.7	4.5	140	29	Poor
III–6	1942	Alfalfa	Wilted, 60% D.M.	None	60.0	57.2	4.5		12	Fair

[a] The first number refers to the silo and the second to the layer. The layers are in the order in which they were removed from the silo.

[b] A mixture of 10 lbs. salt, 36.4 gm. Silogerm, 9.05 gm. lactose, and water to make 120 lbs. was applied at the rate of 30 lbs. per ton as recommended by the manufacturer of Silogerm.

[c] Palatability was judged by odor, appearance and consumption.

use of other adjuvants. As judged by pH, odor, carotene content and palatability, alfalfa ensiled with corn and cob meal, and sweet clover in combination with green corn in the ratios of 1 : 1 and 3 : 1 gave good silages. A fair silage was obtained from wilted alfalfa. Poor silages were obtained from alfalfa ensiled with Silogerm and salt and with Vacatone (residual solids from the industrial fermentation of molasses). The untreated alfalfa was very poor. More extensive chemical analyses were performed on the alfalfa preserved with Silogerm and salt. The ammonia nitrogen in this silage amounted to 0.70 per cent, the acetic acid to 3.32 per cent, and the butyric acid to 4.98 per cent, all calculated on the basis of dry-matter. The volatile acids and the ammonia content of this silage were similar to the values for poor quality silage reported in previous experiments (5).

In 1942 other materials were used as preservatives (layers III–1 to III–6). The object in these experiments was to raise the dry-matter content of the ensiled material to 30 or 35 per cent. The fresh forage was in the late stages of maturity when ensiled. The alfalfa layers preserved with ground or whole corn and by wilting produced fair to good silages. The wilted forage had been rained on twice and had been wilted for 3 days, resulting in a very low carotene content. The alfalfa layers treated with oat straw and ground wood shavings were not palatable and had characteristic straw-like and woody odors. Dry sorghum fodder did not preserve the alfalfa, for the silage was moldy and dry, and the carotene content was low, even though the acidity was equal to that found in good silages.

Composition of silages used in longer feeding trials. Of the many preservatives used in the palatability tests, several were tested further on a larger scale in order to include more complete feeding trials. In 1940, three different silos were filled with the following forages: oat-and-pea mixture sown in a ratio of 4 to 1, Sudan and soybean combination in a seeding ratio of 2 to 1, and alfalfa. Table 2 gives the kind and the amount of preservative used, as well as the analytical data on the fresh forage and the corresponding silage. All three silages were very palatable. However, the carotene contents of lots 3a, 3b, and 3c were low regardless of the kind and amount of preservative used. There was no apparent explanation for the high carotene loss.

In the following year, 1941, the soybean-sorghum silage, in ratios of 1 : 1, 2 : 1, and 4 : 1, was of good quality, although the loss of carotene during ensiling was high in two of the lots. Lots 5a, 5b, and 5c, which were preserved with whey powder, were rated as good silages in spite of the poor carotene preservation. Lots 6a, 6b, and 6c, which consisted of one layer containing no preservative and two others containing different amounts of corn and cob meal, were very good silages as judged by odor and color. However, the carotene preservation was not the same for all layers. The layer which contained 250 pounds of corn and cob meal had very little caro-

TABLE 2

Composition of silages used in feeding trials

Lot	Year	Material	Treatment	Preservative	Dry matter		pH	Carotene (dry basis)		Carotene loss
					Fresh forage	Silage		Fresh forage	Silage	
				lbs., ton	%	%		*µg./gm.*	*µg./gm.*	%
1a	1940	Oats-peas	None		27.8	28.7	4.1	131	99	24
1b	1940	"	Phosphoric acid	15	29.1	27.4	4.0	144	130	10
1c	1940	"	"	8	27.4	25.8	3.9	149	138	7
1d	1940	"	Molasses	40	25.3	25.3	3.9	165	130	21
2a	1940	Sudan (2)– soybeans (1)	None		20.7	19.9	3.9	204	106	48
2b	1940	"	Phosphoric acid	12	20.4	17.4	4.5	266	128	52
2c	1940	"	Molasses	40	20.8	19.6	4.1	220	196	11
3a	1940	Alfalfa	Molasses	60	29.7	27.5	4.4	126	76	40
3b	1940	"	Corn and cob meal	150	30.2	33.8	4.3	160	32	80
3c	1940	"	"	200	28.8	33.5	4.3	188	90	52
4a	1941	Soybeans (1)– sorghum (1)	None		29.7	31.6	3.7	143	84	41
4b	1941	Soybeans (2)– sorghum (1)	None		32.2	30.7	4.0	143	74	48
4c	1941	Soybeans (4)– sorghum (1)	None		34.9	30.1	4.3	98	85	13
5a	1941	Alfalfa	Whey powder	10	27.4	30.6	4.5	135	56	59
5b	1941	"	"	20	28.6	28.0	4.4	135	32	76
5c	1941	"	"	30	34.3	32.7	4.3	135	68	50
6a	1941	"	None		34.6	36.2	4.5	113	7	94
6b	1941	"	Corn and cob meal	200	29.5	29.6	4.3	113	17	85
6c	1941	"	"	250	29.5	28.0	4.3	113	89	21
7	1942	"	Wilted, 40% D.M.		41.7	39.9	4.9	116	64	45
8	1942	"	Corn and cob meal	200	29.5	30.8	4.5	125	70	44
9	1943	Corn	None		22.4	21.7	3.8	107	72	33
10	1943	Alfalfa	Corn and cob meal	200	24.0	23.6	4.4	202	241	0

tene loss; 200 pounds of corn and cob meal gave very low carotene preservation; and the layer which was untreated had lost most of the original carotene. The difference in carotene preservation obtained with a variation of 50 pounds in corn and cob meal was unexpected. The alfalfa used in these experiments was ensiled in the late blossoming stage in order to raise the dry-matter content. In consequence of this more mature condition, it was low in carotene and in most lots the loss was heavy. Corn and cob meal is slow in promoting the development of lactic acid–producing bacteria, which are important in bringing about anaerobic conditions and consequent retardation of carotene oxidation. Also, a high dry-matter content is less favorable for good packing and exclusion of air from the ensiled material. With several factors involved, it is possible that in a given case irregular results may be obtained. Even with molasses, which is a much better preservative than corn and cob meal, poor preservation is encountered occasionally. In our judgment, good preservation of carotene should not entail a loss of more than 25 per cent of the carotene of the forage.

In 1942, two silos were filled with alfalfa preserved by wilting to 40 per cent dry matter (lot 7) and by adding 200 pounds of corn and cob meal per ton (lot 8). Chemical analyses of these two silages (table 2) show that good silages were obtained. The carotene loss was approximately 50 per cent. The alfalfa silage containing corn and cob meal was more readily consumed by the animals than the untreated alfalfa.

In 1943, the experiments were set up to determine the comparative value of corn and alfalfa silages for maintaining the carotene and the vitamin A content of milk. To obtain silages high in carotene content, material in the early stages of maturity was ensiled. In one silo, succulent alfalfa was preserved with 200 pounds corn and cob meal and, in another, corn in the early-dough stage was ensiled. Excellent silages (lots 9 and 10) were obtained. The data show that in order to obtain good silage, the material ensiled must be of good quality.

Milk production and change in body weight. Table 3 summarizes the data on feed consumption, milk production, and changes in body weight of animals fed the silages listed in table 2. In the 1940, 1941, and 1943 feeding experiments, each lot consisted of five cows, while in the 1942 feeding trials the lots were made up of nine cows each. The alfalfa hay used for these experiments contained from 15 to 25 micrograms of carotene per gram of dry matter. The rations for all lots, except 10 and 11, contained equivalent amounts of protein as the result of adding linseed meal to the grain mixtures of lots 1, 2, 4, and 9. From the data in table 3 it appears that the silages were of approximately equal nutritive values, as judged by milk production and body weight.

In 1942 feeding trials showed that apparently all of the corn and cob meal used as preservative for the alfalfa was available to the animal. The

TABLE 3

Feed consumption, milk production and change in body weight per cow per day

Lot	Silage fed	Silage	Alfalfa hay	Grain mixture*	Av. daily 4% F.C. milk	Weight change	Av. decline 4% F.C. milk
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1940	*17 weeks feeding period*						
1	Oats-peas	42.4	9.2	11.3	27.97	+ 0.31	0.101
2	Sudan-soybeans	43.6	9.7	11.2	28.56	− 0.06	0.088
3	Alfalfa–corn and cob meal	42.8	9.5	10.9	28.03	+ 0.56	0.102
1941	*16 weeks*						
4	Sorghum-soybeans	48.8	6.4	8.3	26.85	− 0.029	0.105
5	Alfalfa–whey powder	47.4	6.2	8.3	25.02	− 0.33	0.139
6	Alfalfa–corn and cob meal	49.1	6.4	8.3	26.88	− 0.06	0.139
1942	*7 weeks*						
7	Alfalfa–wilted	31.4	5.6	10.4	30.09	− 0.42	0.066
8	Alfalfa–corn and cob meal	50.1	5.5	6.1	30.16	− 0.07	0.038
1943	*13 weeks*						
9	Corn	40.3	14.0	8.5	25.67	+ 0.80	0.065
10	Alfalfa–corn and cob meal	40.8	14.0	8.7	24.41	+ 0.91	0.080
11	Corn, ½; alfalfa–corn and cob meal, ½	40.4	14.0	8.7	25.02	− 0.86	0.088

* Ingredients	Lots 1, 2 and 4	Lots 3, 5 and 6	Lot 7	Lot 8	Lots 9, 10 and 11
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Ground corn	64.4	69.4	48.5	19.0	38
Ground oats	24.8	27.7	48.5	76.2	30
Bran					20
Linseed meal	9.9				10
Bone meal		1.9	2.0	2.9	
Iodized salt	1.0	1.0	1.0	1.9	1.0
Calcium flour					1.0
Irradiated yeast					0.025

grain mixture fed lot 8 was adjusted to allow for the corn and cob meal used in the preservation of the alfalfa. There were no differences between the two lots, one receiving wilted alfalfa silage (lot 7) and the other silage preserved with corn and cob meal (lot 8).

Lots 9, 10, and 11, which were based on an ordinary farm ration, showed that good corn silage is equivalent to good alfalfa silage for milk production.

Carotene and vitamin A content of milks. The data for the carotene and vitamin A intake of each lot of cows on the feeding trials as well as the vitamin A potency of the milks produced are given in table 4. The milks from the cows in lots 1, 2, and 3 had a high carotene and vitamin A content as compared to the milks produced in the 1941, 1942, and 1943 trials. Milk from lots 4, 5, and 6 had only about 50 per cent of the vitamin A potency (carotene and vitamin A together) of the 1940 milks. The silages fed were low in carotene, and the carotene and vitamin A contents of the corresponding milks were below the average for winter milk. The feeding trials in 1942 showed no differences in the vitamin A potency of the milks produced by animals fed wilted alfalfa silage and those fed alfalfa preserved with corn and cob meal. Waugh *et al* (6), in comparing two groups of cows, one fed corn silage and the other alfalfa–brome grass silage, found no differences in milk production or change in body weight. However, the cows fed corn silage containing 50 micrograms of carotene per gram of dry matter produced milk exceedingly low in vitamin A potency (approximately 9 I.U. per gram of butterfat). The animals fed alfalfa–brome grass silage produced milk of vitamin A potency above the average for winter feeding.

The 1943 experiments indicated that corn silage of high carotene content was almost equivalent to excellent alfalfa silage.[3] Although the cows in lot 10 ingested approximately three times as much carotene as those in lot 9, the vitamin A potency of the milk was only 20 per cent higher than from lot 9 and no higher than that from cows ingesting about two-thirds as much carotene (lot 11). The carotene and vitamin A content of milk from lot 10 accounted for less than one per cent of the carotene intake. In lot 9, 2 per cent of the ingested carotene was accounted for in the milk. As may be seen, the amount of carotene ingested did not correlate with the vitamin A potency of the milk produced. Other undetermined factors appear to have operated. The vitamin A level of the milk for each lot remained constant for the 13 weeks of the 1943 feeding trial.

Survey of experimental farm milks. These data were obtained as part of a grass silage harvester program under farm conditions which was sponsored

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[3] Another short experiment, in which cows fed a low-carotene corn silage (26 μg. per gram dry matter) were switched to a high-carotene corn silage, showed that the vitamin A potency of the milk followed the increase in carotene intake very closely. At the beginning of the experiment the vitamin A potency of the milk was 14 I.U. per gram of butterfat and, after feeding high carotene silage for 3 weeks, it rose to 30 I.U. per gram of butterfat.

TABLE 4

Carotene and vitamin A content of milks produced in feeding trials

Lot	Year	Silage	Butterfat	Carotene intake*	Carotene	Vitamin A	Vitamin A potency, I.U.	
			%	*mg./day*	*µg./gm. butterfat*	*µg./gm. butterfat*	*per gm. butterfat*	*per 100 ml. milk*
1	1940	Oats-peas	3.5	722	9.3–10.2 9.8†	8.3–8.6 8.5†	50	183
2	1940	Sudan-soybean	3.5	641	8.5–8.9 8.7	7.1–8.3 7.7	45	166
3	1940	Alfalfa–corn and cob meal	3.9	491	6.0–7.5 7.3	8.2–8.6 8.4	46	185
4	1941	Soybean-sorghum	3.9	611	2.1–3.4 2.9	2.9–5.2 3.9	20	84
5	1941	Alfalfa–whey powder	3.5	396	3.3–3.7 3.5	4.2–4.6 4.4	23	83
6	1941	Alfalfa–corn and cob meal	3.8	323	1.5–3.0 2.5	2.3–4.9 3.6	19	74
7	1942	Alfalfa–wilted, 40% D.M.	3.55	415	2.9–3.2 3.0	6.1–6.6 6.4	31	113
8	1942	Alfalfa–corn and cob meal	3.85	540	5.2–5.6 5.4	5.9–6.0 6.0	33	131
9	1943	Corn	4.0	414	4.1–5.8 4.6	5.2–7.4 5.6	30	124
10	1943	Alfalfa–corn and cob meal	3.6	1164	4.2–7.0 5.7	6.0–7.9 6.9	37	137
11	1943	Corn (1 part); alfalfa and cob meal (1 part)	4.2	784	3.8–5.9 5.4	5.2–6.5 5.9	33	143

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* Includes carotene supplied by hay; 20 µg. per gram of dry matter was used as an average value.

† The third figure is the average of the preceding two figures.

TABLE 5

Data on experimental farm silages and milks

Farm no.	Silage*	Silage analysis			Milk analysis			Vitamin A potency, I.U.	
		pH	Dry matter	Carotene	Butterfat	Carotene	Vitamin A		
			%	*µg./gm. dry matter*	%	*µg./gm. butterfat*	*µg./gm. butterfat*	*per gm. butterfat*	*per 100 ml. milk*
1	Alfalfa	4.2	29.5	250	3.5	4.7	10.6	50	180
2	Sweet clover	3.9	29.5	75	3.7	3.4	7.0	34	130
3	Alfalfa	4.1	26.3	154	3.9	3.8	5.9	30	120
4	Alfalfa	4.2	29.3	156	4.8	4.8	5.6	30	148
5	Alfalfa	4.4	30.5	30	3.2	2.1	5.7	26	86
6	Timothy	4.1	29.3	74	3.6	1.6	5.3	24	89
7a	Corn				5.0	8.8	3.0	27	139
7b	Alfalfa		25.6	118	4.7	10.0	3.4	30	145

* All grass silages were preserved with 200 lbs. corn and cob meal with the exception of that from farm no. 4 which was preserved with molasses.

by the College of Agriculture. The analyses (table 5) show that four of the alfalfa silages were unusually high in carotene. These were ensiled during the early stages of maturity. Farm no. 1 produced milk which had a vitamin A potency equal to that of summer milk. This herd had been fed high carotene silage for 3 months previous to the time the sample was taken. It will be noted that the feeding of low-carotene silage (*e.g.*, farm no. 5) resulted in milk low in vitamin A potency.

An experiment was conducted at the La Crosse, Wisconsin, Soil Conservation Experiment Station (farm no. 7) to note the effect of replacing corn silage in an ordinary farm ration with alfalfa silage. During the last month (February) on corn silage, the vitamin A potency decreased from 32 to 27 I.U. per gram of butterfat. The cows were then switched to alfalfa silage which contained 118 micrograms of carotene per gram of dry matter. The decline stopped, and after 3 weeks the vitamin A potency reached 30 I.U. per gram of butterfat and remained at this level for a month, when the experiment was terminated.

SUMMARY

In a study of various methods of ensiling grasses and legumes, preservation by the addition of 200 pounds corn and cob meal per ton gave a very palatable silage, although carotene preservation was not as good as with some other methods, *e.g.*, molasses.

No significant differences were found in milk production and change in body weight when the lots for any one feeding period were compared.

A comparison of good corn and alfalfa silages indicated that both silages had apparently equivalent feed value on the basis of milk production and change in body weight. The vitamin A potency of the milk produced from these two silages varied by only 20 per cent. Alfalfa silage high in carotene generally increased and maintained the carotene and vitamin A level in winter milk.

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THE EFFECT OF SUPPLEMENTARY VITAMINS ON BLOOD COMPOSITION, LIVER STORAGE, AND INCIDENCE OF SCOURS IN CALVES

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Following the reports by Wisconsin workers (4, 8) that supplementary vitamin feeding is beneficial in preventing calf scours, much interest has developed regarding the possibilities in vitamin supplementation for calves. Most previous work has been done with calves maintained on skim milk or calves from dams known to be on a low level of vitamin A intake. We, therefore, undertook to determine the effect of two systems of vitamin supplementation on the blood picture, liver storage, and scour incidence of calves maintained under normal herd conditions.

In these experiments each calf was allowed to nurse before the dam was milked out. The calves remained on their dams for at least 3 days and were then pail-fed on whole milk. Hay and grain were fed beginning at 2 weeks of age. Calves of both the Jersey and Holstein breeds were included in this experiment and the blood and liver data of both breeds are combined in the results.

Blood plasma vitamin A and carotene were determined by the method of Kimble (2). Liver vitamin A and carotene were determined by using the extraction procedure of Guilbert and Hart (1). Blood plasma ascorbic acid was determined by the macromethod of Mindlin and Butler (6). All colorimetric readings were made using an Evelyn photoelectric colorimeter.

EXPERIMENT 1

The first experiment was carried out during the late winter and early spring months of 1945, before the pasture season. Alternate calves were placed in Group I (control) and Group II (experimental). Group I (fifteen calves) received a placebo capsule[1] containing a biologically inactive oil. Group II (fifteen calves) received one multivitamin capsule daily for the first 20 days. These capsules contained 10,000 USP units of vitamin A, 300 USP units of vitamin D, 50 mg. of niacin, and 250 mg. of ascorbic acid.

Blood samples were drawn for analysis of plasma vitamin A, carotene, and ascorbic acid on the same day each week from all calves under 31 days of age. In averaging the results, all determinations made on the first, second, and third days were averaged separately. All determinations made between the fourth and eleventh days were grouped together and considered as the seventh

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[1] All vitamin capsules were supplied by The Gelatin Products Company, Detroit, Michigan, to whom grateful acknowledgment is herewith extended.

day. Similar groupings of the data were made centering on the fourteenth, twenty-first, and twenty-eighth days. Total vitamin A liver storage was determined on four male calves in Group I and on five male calves in Group II at 21 days of age.

The average results of the blood and liver analyses are presented in figure 1. The vitamin A liver storage indicated is due to vitamin A alone. A small carotene storage was found which amounted to an average of 813 USP units per liver in the nine calves. Plasma vitamin A reached a peak on the third day and then declined rapidly. No difference in the groups was observed until after the third day, when it was shown that Group II (experimental) did not decline in blood vitamin A as rapidly as did Group I (control). There was a decline in blood vitamin A regardless of supplementary feeding. Liver storage at 21 days in Group II was found to be nearly double that of Group I, indicating marked liver storage due to the supplemental vitamin A. Greater individual variations were found in the plasma carotene levels; however, no marked difference between the two groups is indicated.

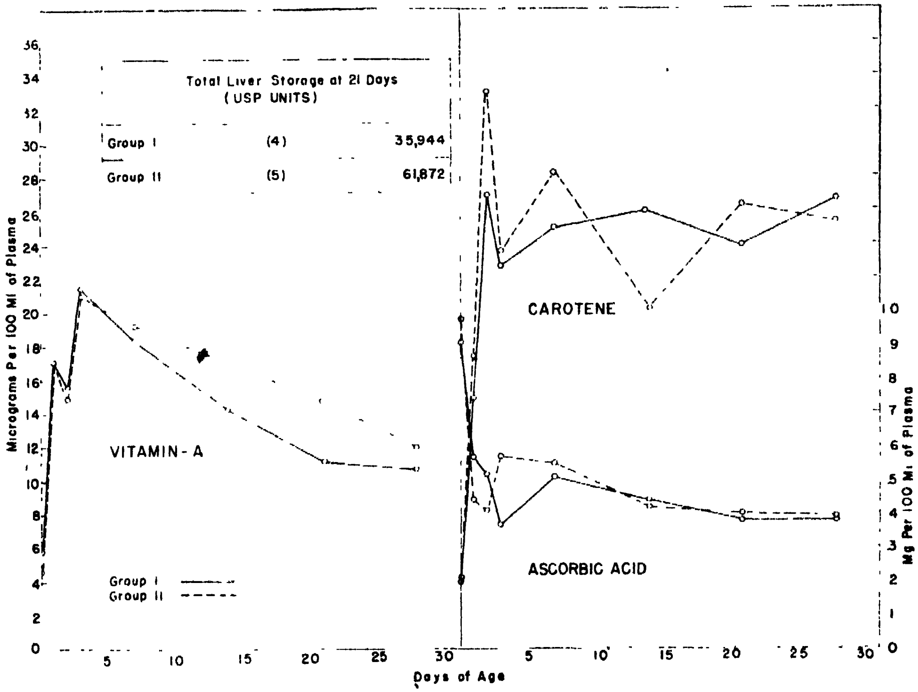

FIG. 1. The effect of daily supplementary vitamin feeding on the blood plasma vitamin A, carotene, and ascorbic acid, and on vitamin A liver storage in calves.

Plasma ascorbic acid was found to be extremely high immediately after birth. The initial high level rapidly dropped so that normal levels were usually found within 24 hours. No beneficial effect of feeding ascorbic acid was observed except that Group II showed a slight increase over Group I between the third and seventh days.

Although a considerable number of calves had scours, no difference was noted in the incidence between Group I and Group II.

EXPERIMENT 2

To determine the effects of feeding massive doses of vitamin A at less-frequent intervals, the following experiment was carried out from February through April, 1946. Holstein and Jersey calves born in the Experiment Station herds were assigned to one of three groups. Group I (ten calves) served as a control group. Group II (thirteen calves) received a capsule containing 250,000 USP units of vitamin A on the third and tenth days after birth. Group III (twelve calves) received the same vitamin A supplement and, in addition, were given 50 mg. of niacin in a gelatin capsule daily for the first 20 days. Blood samples were drawn for analysis for plasma vitamin A, carotene, and ascorbic acid on the third, tenth, and twentieth days for all calves and also on the thirtieth day for the females. The male calves in each group were sacrificed on the twenty-first day and the total vitamin A liver storage determined. The average results of the blood and liver storage data are shown in figure 2.

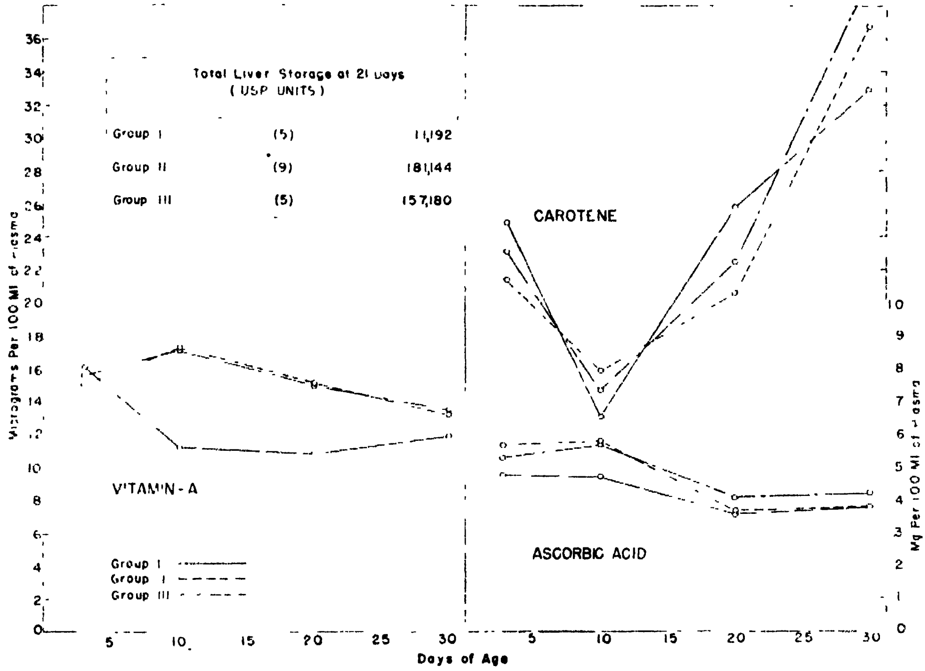

FIG. 2. The effect of feeding 250,000 USP units of vitamin A on the third and tenth days, plus 50 milligrams of niacin daily for 20 days, on the blood plasma vitamin A, carotene, and ascorbic acid, and on liver storage in calves.

It is evident that the supplemental vitamin A feeding resulted in the maintenance of a higher blood level in Groups II and III. However, the most marked effect of vitamin A feeding showed up in the amounts stored in the liver. Nearly ten times as much vitamin A was stored in the livers of the experimental group as in the control group. No difference was noted between Groups II and III in the blood level of vitamin A or in liver storage. Thus, the addition of niacin did not raise the blood level of vitamin A or increase liver storage at this level of vitamin A intake. The average liver carotene value of the nineteen male calves in the three groups was 633 USP units per liver.

Although the blood carotene followed a somewhat different pattern in experiment 2 than in experiment 1, no difference among the three groups is detectable. No differences were noted among the groups regarding the average ascorbic acid level. The incidence of scours was the same in all three groups.

DISCUSSION

In these experiments, no lowering of scours incidence was noted which could be attributed to supplemental vitamin feeding. This is in general agreement with the work of Norton *et al.* (7).

The incidence of scours was much higher in the Jersey calves than in the Holsteins. The average plasma vitamin A of the Jersey calves was lower than that of the Holsteins. Possibly this is due to the higher incidence of scours in the Jersey calves. The average carotene level of Holstein plasma was lower than that of the Jerseys. This observation is in agreement with the results reported by Moore (5). Plasma vitamin A and carotene, as well as vitamin A liver storage, were reduced in calves that had severe scours.

These data show that the decrease in plasma vitamin A of calves during the first few weeks can be offset to a considerable extent by feeding supplemental vitamin A according to either of these two systems. It is of interest that Sutton and Kaeser (9) have shown that, when colostrum feeding was extended for 7 days, the blood vitamin A level at 21 days was nearly identical with that of calves that received 10,000 units of vitamin A daily for 21 days.

No linear correlation between plasma vitamin A and liver storage was observed when liver storage was high. At low liver storage levels the plasma vitamin A values were a good index of liver storage. In general, data reported by Lewis and Wilson (3) confirm these observations. However, the experimental procedures are not sufficiently comparable for direct comparison of the data.

It is questionable how much benefit to the health of the calf is derived from excessively high liver storage or increases in blood vitamin A over the normal levels, when a normal ration is fed. In large-scale field trials conducted in Ohio and Michigan, the results of which are to be published elsewhere, no lowering of scour incidence could be attributed to the feeding of supplemental vitamin A. It is reasonable to believe, however, that calves with a high vitamin A storage would be able to withstand periods of low vitamin A intake or impaired absorption much better than calves raised without supplementary vitamin A. Since both the plasma vitamin A content and liver storage of calves that had severe scours were extremely low, the administration of supplementary vitamin A to such calves would seem to be indicated in order to counteract the subnormal levels. Calves with severe scours often had plasma vitamin A levels as low as 4.5 micrograms per 100 ml. In Group III one calf, which had severe scours for 8 days, had a vitamin A liver storage value of 54,420 USP units at 21 days, whereas the average value for the group was 157,180 USP units. This calf had a vitamin A blood plasma level of 4.4 micrograms per 100 ml. at 20 days of age. The average blood vitamin A level for the group at this age was 13.9 micrograms per 100 ml.

SUMMARY AND CONCLUSIONS

1. Calves fed extra vitamin A, either 10,000 USP units daily for 20 days or 250,000 USP units on the third and tenth days, maintained a higher blood plasma vitamin A level after the third day than did their controls.

2. Liver vitamin A storage was increased with increased vitamin A intake. Plasma vitamin A and liver storage were not closely correlated except at low levels of liver storage.

3. The daily addition of 50 mg. of niacin when 250,000 USP units of vitamin A were fed on the third and tenth days had no effect on blood plasma or liver storage vitamin A values.

4. Except for a slight increase between the third and seventh days, no effect was observed on the plasma ascorbic acid content when 250 mg. of ascorbic acid were fed daily for 20 days.

5. No significant effect on lowering the incidence or severity of scours could be detected when supplementary vitamins were added to the normal ration according to the procedures described.

It is concluded that routine supplementary feeding of vitamin A, ascorbic acid, and niacin to calves during the first few weeks following birth is of doubtful value in preventing scours. The feeding of supplementary vitamin A at the rate of 10,000 USP units daily for 20 days or 250,000 USP units on the third and tenth days will help overcome any deficiency of vitamin A intake resulting from inadequate feeding of colostrum and whole milk, from impaired absorption, or from subsequent feeding of poor-quality hay.

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COPPER AND IRON IN THE BLOOD SERUM OF DAIRY COWS[1, 2, 3]

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The usefulness of blood or blood serum levels of copper, iron, hemoglobin, and cell volume as indications of nutritional deficiencies of cattle grazing in areas deficient in certain micronutrient elements has been shown by the work of various investigators (1, 2, 3, 5, 13). The utility of this blood picture tool, however, is dependent on a knowledge of the normal levels and variabilities of these blood characteristics. A review of the literature reveals no general agreement in the results reported for blood serum copper and iron levels for dairy cows. In view of this, the work reported here was undertaken as preliminary to a project designed to study certain deficiencies of micronutrient elements in some coastal areas of North Carolina. Hemoglobin and cell volume were run concurrently with iron and copper and also are reported.

Most of the published copper values on bovine blood are reported on whole blood rather than on blood serum. Tompsett (16), however, presented data showing that the copper of the blood was distributed evenly between the plasma and the corpuscles of the blood for man, sheep, ox, pig, and horse. The data of Kehoe *et al* (9) also show the copper content of the corpuscles and plasma of blood to be of the same order. In this laboratory, no significant difference was found in copper content between blood serum badly contaminated with red cells and serum with little or no red-cell contamination. The copper content for apparently normal bovine blood or blood serum, as determined by various workers, is shown in table 1. Blood serum iron values for dairy cows are meager.

MATERIALS AND METHODS

The principal difficulty in determining iron in blood serum is to obtain a preparation completely free from hemoglobin and other forms of organic iron. This problem has been discussed by Kitzes *et al* (10), and a method is presented which appears to give satisfactory results. In our work, the method of Kitzes was used to prepare the blood serum filtrate for the iron

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determination. Instead of a,a′-bipyridine, however, *o*-phenanthroline was used for the color reagent, as outlined by Parks, *et al* (14).

In some preliminary work, it was found that wet-ashed serum gave higher copper values than blood serum filtrate prepared according to Kitzes. Typical values obtained were: wet-ashed serum, 103 µg. of copper per 100 ml. of serum; and blood serum filtrate, 68 µg. of copper per 100 ml. of serum These values are an average of twelve samples in duplicate. In view of these results, a wet-ash method was adopted. The blood serum was wet-ashed with concentrated nitric and perchloric acid. Essentially the method outlined by Parks *et al* (14) was used for the determination of copper. The method of Shenk *et al* (15) was used for the hemoglobin determination, and the Sanford Magath hematocrit tube for cell volume.

The experiment was designed to study the influence of breed, age, and time on the levels of the blood constituents under study. Blood was obtained from Ayrshire, Holstein, Guernsey, and Jersey cows in the college herds. The animals were subdivided into four age classifications: 4 to 6 months, 12 to 18 months, 2 to 2.5 years, and 4 to 7 years, with one animal from each breed in each age class. Blood from each animal was analyzed at four different times: January 2, February 14, March 6 and March 20. The four different dates will be referred to as Periods I, II, III, and IV, respectively. The cows were barn-fed for all periods except Period IV, when they were on pasture. Throughout the experimental period, all the animals received hay and a 17 per cent protein concentrate mixture. In addition, the animals in the age groups 2 to 2.5 years and 4 to 7 years received corn silage. The calves, 4 to 6 months of age, received a supplement of milk during most of the first two periods and a supplement of calf manna during the remainder of the experiment.

TABLE 1

Normal bovine blood or blood serum values obtained by various workers

Author	Animal	Type of blood	Copper
			µg./100 ml.
McHargue (12)	Ox	Whole blood	140
Guillemet (6)	Cow	Serum	58–82
Tompsett (16)	Ox	Whole blood and serum	192–223
Bennetts *et al* (3)	Cow	Whole blood	30–100
Beck (2)	Cow	Whole blood	70–170
Cunningham (5)	Cow	Whole blood	Mean of 100

EXPERIMENTAL DATA

The means for iron, copper, hemoglobin, and cell volume, classified according to the ages of the animals, together with their standard errors, are presented in table 2.

TABLE 2

Effect of age on the blood level of indicated characteristic

Blood characteristics	Age				Standard error of a mean $S_{\bar{x}}$	Significance of age effect*
	4–6 months	12–18 months	2–2½ years	4–7 years		
Serum iron, µg./100 ml.	171.2	155.0	157.3	162.4	± 12.0	o
Serum copper, µg./100 ml.	91.5	85.0	109.9	113.8	± 6.2	hs
Hemoglobin, gm./100 ml.	9.08	9.72	10.06	10.64	± 0.26	hs
Cell volume, % of total	29.37	30.92	32.40	34.53	± 1.27	o

* o = not significant ($P > 0.05$).
s = significant ($P < 0.05$).
hs = highly significant ($P < 0.01$).

As indicated in the table, no evidence was obtained that serum iron changes with age. Copper and hemoglobin, however, changed significantly with age, the higher values being observed with advancing age. Although cell volume increased with advancing age, this effect was not statistically significant. On the other hand, there was a positive and significant correlation ($r = +0.9986$) between hemoglobin and cell volume from age group to age group. It might be concluded, therefore, that perhaps both hemoglobin and cell volume increase with advancing age.

The influence of breed on the blood levels of the characteristics studied is summarized in table 3.

No evidence was obtained that the blood characteristics differ among breeds. In this connection Tompsett (16), in his copper studies of various species, reports "the copper contents of sheep, ox, pig, horse, and guinea pig are of the same order as that of human blood." McCay (11) and Brooks *et al* (4), working with the four breeds used in this study, also found no difference in hemoglobin levels among breeds.

The effect of period on the blood levels of the characteristics is presented in table 4.

TABLE 3

Effect of breed on the blood level of the indicated characteristic

Blood characteristics	Breed				Standard error of a mean ($S_{\bar{x}}$)	Significance of breed effect*
	Ayrshire	Holstein	Guernsey	Jersey		
Serum iron, µg./100 ml.	130.8	168.3	163.7	183.1	± 12.0	o
Serum copper, µg./100 ml.	93.6	92.5	99.1	115.6	± 6.2	o
Hemoglobin, gm./100 ml.	10.39	9.50	9.94	9.67	± 0.26	o
Cell volume, % of total	32.14	29.78	34.45	30.85	± 1.27	o

* o = not significant ($P > 0.05$).

TABLE 4

Effect of period on the blood level of the indicated characteristics

Blood characteristics	Period				Standard error of a mean ($S\bar{x}$)	Significance of period effect*
	I 1/2/46	II 2/14/46	III 3/6/46	IV 3/20/46		
Serum iron, µg./100 ml.	130.3	169.2	162.6	183.9	±6.8	hs
Serum copper, µg./100 ml.	87.8	101.2	116.3	95.2	±4.6	hs
Hemoglobin, gm./100 ml.	10.07	9.93	10.06	9.44	±0.17	s
Cell volume, % of total		30.94	31.54	32.94	±0.75	o

* o = not significant ($P > 0.05$).
s = significant ($P \leq 0.05$).
hs = highly significant ($P \leq 0.01$).

As illustrated by table 4, period had a pronounced effect on all measures except cell volume. Possibly, if cell volume had been measured in Period I, it also would have shown a significant period variation. This experiment was not designed to sort out and find the causes of this period variation. Presumably, however, such factors as nutritional status of the animal, season of the year, and post-absorptive state of the animal are contributing to period variations. For example, Hemmeler (8) reports that in humans the serum iron is highest in the morning, averaging 127 µg. per 100 ml , and lowest in the evening, averaging 82 µg. per 100 ml. Hazleton (7) administered single doses of iron salts to rats and found the maximal level of serum iron to occur two to three hours after administration. Bennetts *et al* (3) made monthly analyses of blood copper of healthy cows from April through December and reported a low of 30 to 50 µg. in April and a high of 60 to 100 µg. per 100 ml. in September.

In table 5 are presented the over-all means and their coefficients of variation. It may be pointed out that the observations which make up the general or over-all means are subject to both the controlled variation or experimental error due to technique, age, breed, and period, as well as uncontrolled variation. The coefficients of variation presented in table 5 are measures of total variation; they include unbiased estimates of both controlled and uncontrolled variation for dairy cows under the conditions of the experiment.

The error due to technique, such as sampling, manipulation, reading, etc.,

TABLE 5

Grand means of the indicated blood characteristics and their coefficients of variation

Blood characteristic	Grand mean	Coefficient of variation
		%
Serum iron, µg./100 ml.	161.5	26.75
Serum copper, µg./100 ml.	100.1	23.93
Hemoglobin, gm./100 ml.	9.87	10.20
Cell volume, % of total	31.8	13.07

was less than 5 per cent for serum iron and copper and less than 2 per cent for hemoglobin.

No evidence was obtained that iron and copper are correlated in any way. On the other hand, hemoglobin and cell volume, in general, were correlated positively. The only deviation from this observation was the negative correlation which existed among periods (– 0.8812). This negative correlation was not significant, but suggested that the factors causing the blood picture to change from period to period affect cell volume and hemoglobin in opposite manners.

SUMMARY

1. The means for serum iron, serum copper, hemoglobin, and cell volume in sixteen dairy animals were 100 µg. per 100 ml., 162 µg. per 100 ml., 9.9 gm. per 100 ml., and 32 per cent of total volume, respectively. The errors of estimate are given.

2. No evidence was obtained that serum iron changed with age. Serum copper and hemoglobin changed significantly with advancing age. The copper values ranged from about 90 µg. per 100 ml. in calves to 114 µg. in 4- to 7-year-old cows, and the hemoglobin increased from 9.08 to 10.64 gm. per 100 ml. of blood in these age classes.

3. There was no significant difference among breeds with respect to any of the measurements.

4. For serum iron, serum copper, and hemoglobin, there were pronounced variations from period to period. These variations were irregular with ranges as follows: serum iron, 130.3 to 183.9 µg. per 100 ml.; serum copper, 87.3 to 116.3 µg. per 100 ml.; and hemoglobin, 9.44 to 10.07 gm. per 100 ml.

5. No evidence was obtained that serum iron and serum copper are correlated in any way. Hemoglobin and cell volume, in general, were correlated positively.

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COMPARISON OF SUCROSE, HIGH CONVERSION CORN SIRUP, AND DEXTROSE IN THE PRESERVATION OF PEACHES BY THE FROZEN-PACK METHOD FOR USE IN ICE CREAM

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The use of frozen-pack fruits in the ice cream industry has been gaining in popularity in recent years. This has been due largely to the superior flavor of the frozen fruit as compared with the canned. The favorable experiences with frozen foods gained during World War II undoubtedly will place even greater emphasis upon the method of preserving and transporting fruits in frozen form.

While many ice cream manufacturers prepare much of their own pack of peaches, a considerable portion of their supply is purchased from commercial dealers. The peeled peaches are sliced or made into puree and mixed with sugar (cane or beet) in varying proportions, though three parts of fruit to one part of sugar commonly is used. This mixture then is placed in containers, rapidly frozen and stored at temperatures below zero degrees Fahrenheit until used. The sugar shortage during World War II, however, made it important that some consideration be given to the use of other types of sweetening agents, such as dextrose (corn sugar) and corn sirup, in the preparation of frozen fruit to be used in ice cream.

PROCEDURE

The studies were made during late 1941 and early 1942. The fruit was obtained from the peach-breeding plots of the University of Illinois experimental farms at Olney, Illinois, and represents the 1941 crop. The types chosen for study, as listed in table 1, are eight of the most promising selections in the Station peach-breeding project. Observations were made of the flavor, degree of ripeness, and texture of the fruit when received. The peaches were immersed in boiling water for a period of 30 seconds, followed by dipping into cold water. After the fruit skins were removed and the

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peaches halved and stoned, the fruit was immersed in ice water until packing, which was accomplished within 1 hour after the hot-water immersion.

The fruit was packed in pint paper cartons, using various combinations and types of sugar. Immediately after packing, the cartons were placed in a 40° F. room for a period of 2 hours; the fruit was shaken at intervals to aid in the blending of sugar and fruit. The cartons of fruit then were stored at a temperature of approximately −2° to −20° F. for periods of 4 to 8 months. At the end of the storage period, the samples were removed to a 40° F. room and permitted to thaw slowly, after which they were judged for color, flavor, texture, and sugar crystallization.

TABLE 1

Varieties, flavor and degree of ripeness of peaches when packed

Variety (cross)	Flesh color	Quality	Acid*	Degree of ripeness	Date ripe
Heath x Marigold–K ..	White	Good		Slightly green	Aug. 5
Heath x Marigold	White	Good		Slightly green	Aug. 4
J. H. Hale x Gage–K50	Yellow	Good	1.65	Slightly green	Aug. 15
Elberta x Ea. Elberta	Yellow	Very good	0.48	Well ripened	Aug. 22
J. H. Hale x Gage	Yellow	Very good	0.76	Well ripened	Aug. 22
Gage x Elberta	Yellow	Excellent	0.63	Very ripe	Aug. 21
J. H. Hale x Elberta	Yellow	Good	0.72	Well ripened	Aug. 28
Elberta x Southhaven	Yellow	Good	0.49	Very ripe	Sept. 1

* Data on acid content supplied by Dr. R. V. Lott. Values represent percentage malic acid in the fruit juice.

Cane sugar, enzyme converted corn sirup,[3] and dextrose hydrate were the sweetening agents used in the preparation of the experimental packs.

EXPERIMENTAL RESULTS

Heath x Marigold–K

This variety of peach is white. It was packed using fruit-sugar concentrations of (2 + 1), (2.5 + 1), and (3 + 1). The following combinations of sweetening agents were used in the three concentrations listed above:

1. 100 per cent sucrose
2. 100 per cent dextrose
3. 100 per cent enzyme converted corn sirup
4. 25 per cent sucrose plus 75 per cent dextrose
5. 50 per cent sucrose plus 50 per cent dextrose
6. 75 per cent sucrose plus 25 per cent dextrose
7. 25 per cent sucrose plus 75 per cent corn sirup
8. 50 per cent sucrose plus 50 per cent corn sirup
9. 75 per cent sucrose plus 25 per cent corn sirup
10. 25 per cent dextrose plus 75 per cent corn sirup

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[3] This product will be referred to as corn sirup, and contained approximately 18% moisture, 10% dextrins, 41% maltose and higher sugars, and 31% dextrose.

11. 50 per cent dextrose plus 50 per cent corn sirup
12. 75 per cent dextrose plus 25 per cent corn sirup

The peaches were slightly green but had a fair flavor when packed. The fruit was judged after approximately 5 months of storage.

The (2+1), (2.5+1), and (3+1) packs were placed in that order when judged for color. Of the packs prepared with a single type of sweetening agent, the sucrose samples had the best color, the packs prepared with corn sirup were second best, and the dextrose samples had the least desirable color, being very brown. The color of the peaches using the different combinations of sucrose and corn sirup was about the same, though some preference was shown for those packs prepared with 50 per cent corn sirup and 50 per cent sucrose in a concentration of two parts of fruit to one part of sugar.

The best-flavored packs were the (2+1), (2.5+1), and (3+1) in that order. Of the packs prepared with a single sweetening agent, the sucrose-packed fruits were best, the corn sirup packs were second best, and the dextrose-packed samples were third. Considering all lots, the samples having the best flavor contained 50 per cent corn sirup plus 50 per cent sucrose in a concentration of two parts of fruit to one part of sugar.

Dextrose was most soluble in the (3+1) packs. It was less soluble in combination with corn sirup than with sucrose. There was evidence of crystallization of the dextrose in all of the corn sirup and dextrose combinations.

Heath Marigold

This variety of peach was packed using fruit-sugar concentrations of (2+1), (2.5+1), and (3+1). The same combinations of sucrose, dextrose, and corn sirup were used as in the previous experiment.

The (2+1), (2.5+1), and (3+1) packs were placed in that order when judged for color. The corn sirup pack had the best color of all lots containing a single sweetening agent, followed by the sucrose pack and the dextrose pack. The all-dextrose pack was very brown. The colors of the peaches in combinations of corn sirup and sucrose were about the same except the 50 per cent sucrose and 50 per cent corn sirup sample in a (2.5+1) concentration, which was the best of all packs, followed by the same sweetening agent combination in a (2+1) pack.

The best-flavored packs were the (2+1), (2.5+1), and (3+1), in that order. Of the packs containing a single sweetening agent, the sucrose-packed fruits were best, followed by corn sirup and dextrose sugar packs in the order named. The best-flavored fruit of the entire pack was the 75 per cent sucrose plus 25 per cent corn sirup sample in a (2.5+1) concentration, followed by the sample containing 25 per cent sucrose plus 75 per cent corn sirup in a (2+1) concentration. There was evidence of crystallization in all packs where dextrose was used.

Gage × Elberta

This variety of peach was packed using fruit-sugar concentrations of (2 + 1), (3 + 1), and (4 + 1).

The sweetening agents used in this pack were added in the form of sirup and were compared with samples packed using sugar and corn sirup added on a weight basis as follows:

1. 100 per cent sucrose
2. 100 per cent corn sirup
3. 50 per cent corn sirup plus 50 per cent sucrose
4. 40 per cent corn sirup
5. 50 per cent corn sirup
6. 60 per cent corn sirup
7. 40 per cent sucrose sirup
8. 50 per cent sucrose sirup
9. 60 per cent sucrose sirup
10. 20 per cent sucrose sirup plus 20 per cent corn sirup
11. 25 per cent sucrose sirup plus 25 per cent corn sirup
12. 30 per cent sucrose sirup plus 30 per cent corn sirup

These peaches were very ripe and had an excellent flavor when packed. The fruit was judged after 4 months of storage.

The (2 + 1), (3 + 1), and (4 + 1) packs were placed in that order when judged for color. In those samples packed with sirup, the best-colored fruits were obtained using sirups of high sugar concentrations. Of the samples containing a single sweetening agent, the sucrose pack had the best color, followed by the 100 per cent corn sirup pack. Both packs were of excellent color, however.

The best-flavored packs were those of a (2 + 1) concentration, followed by the (3 + 1) and the (4 + 1) packs. The (4 + 1) packs all were bitter. The all-sucrose pack had the best flavor, followed by the 50 per cent sucrose plus 50 per cent corn sirup pack, which was second, and the 100 per cent corn sirup pack, which was third. Of the packs using sirup, the best-flavored samples were prepared with sirups of high (60 per cent) sugar concentration.

J. H. Hale × Gage

This variety of peach was packed using fruit-sugar concentrations of (1 + 1), (2 + 1), (3 + 1), and (4 + 1). The sweetening agents used were added in the form of a 50 per cent sirup and were compared with both dry sucrose and 100 per cent corn sirup as follows:

1. 100 per cent sucrose—dry
2. 100 per cent corn sirup

3. 50 per cent corn sirup plus 50 per cent sucrose (heated to boiling and cooled before adding to peaches)
4. 50 per cent corn sirup plus 50 per cent sucrose (mixed cold before adding)
5. 50 per cent corn sirup plus 50 per cent sucrose (mixed as added to peaches)

These peaches were well ripened when packed and had a good flavor. The fruit was judged after 5 months of storage.

The pack in general had a good appearance, with the color becoming slightly less desirable as the concentration of sugar sirup decreased. The all-sucrose pack had the least desirable color. The (1 + 1) pack resulted in a slightly pulped fruit. All of the samples lacked flavor, with no distinct differences noticeable. In general the pack was not satisfactory.

Elberta × *Early Elberta*

This variety of peach was packed using fruit-sugar concentrations of (1 + 1), (2 + 1), (3 + 1), and (4 + 1). The sweetening agents used were added in the form of sirup or on a dry basis in the following combinations:

1. 100 per cent sucrose—dry
2. 100 per cent corn sirup
3. 50 per cent corn sirup plus 50 per cent sucrose (heated to boiling and cooled before adding)
4. 50 per cent corn sirup plus 50 per cent sucrose (mixed cold before adding)
5. 50 per cent corn sirup plus 50 per cent sucrose (mixed as added)

These peaches were well ripened and had an excellent flavor when packed. The fruit was judged after 6 months of storage.

The 100 per cent corn sirup packs had the best color, followed by 100 per cent sucrose, and 50 per cent corn sirup plus 50 per cent sucrose combinations. The boiled sirup combinations were the least desirable. The natural fruit color increased with the increase in sugar concentrations throughout the pack.

The body and texture of the peaches became less desirable with a decrease in the sugar concentrations, except the (1 + 1) pack, in which the fruit was pulped and the sugar was crystallized.

The best-flavored groups were the packs containing 50 per cent sucrose plus 50 per cent corn sirup, mixed before adding, followed by the packs prepared with 100 per cent corn sirup, 100 per cent sucrose, and 50 per cent corn sirup and 50 per cent sucrose mixed as added. The boiled sirup samples were the least desirable. The intensity of the fruit flavor increased with the increased sugar concentrations. The best individual sample was the (1 + 1) pack using 100 per cent corn sirup, followed by the (2 + 1) concentration prepared with 100 per cent sucrose, and the sample containing 100 per cent corn sirup in a (3 + 1) pack.

The same results were obtained using the same types of packs with a J. H. Hale and Gage-K50.

Elberta × Southhaven

These peaches were packed in 1-gallon paper containers using fruit-sugar concentrations of (2 + 1), (3 + 1), and (4 + 1). The sweetening agents used were 80 per cent sucrose sirup, 80 per cent corn sirup, and 70 per cent corn sirup.

The fruit was well ripened when packed and had an excellent flavor.

The different packs all were judged to be excellent in color with no distinct difference in appearance. All samples also rated excellent in flavor, with the (2 + 1) pack using 80 per cent sucrose sirup best, the (2 + 1) 70 per cent corn sirup pack second, and the (3 + 1) 80 per cent corn sirup pack last.

J. H. Hale × Elberta

This variety of peach was packed using fruit-sugar concentrations of (2 + 1), (3 + 1), and (4 + 1). The sugars used in this pack were added in the form of sirup or on a dry basis in the combinations given below:

1. 100 per cent sucrose
2. 100 per cent corn sirup
3. 50 per cent corn sirup plus 50 per cent sucrose
4. 40 per cent corn sirup
5. 50 per cent corn sirup
6. 60 per cent corn sirup
7. 40 per cent sucrose sirup
8. 50 per cent sucrose sirup
9. 60 per cent sucrose sirup
10. 40 per cent sirup—half sugar and half corn sirup
11. 50 per cent sirup—half sugar and half corn sirup
12. 70 per cent sirup—57 per cent sugar and 43 per cent corn sirup

These peaches were ripe and had a good flavor when packed. The fruit was judged after 4 months of storage.

The (2 + 1), (3 + 1), and (4 + 1) packs were placed in that order when judged for color. The best colors were evident when sirups of high sugar concentrations were used. Of the lots containing a single sweetening agent, the corn sirup pack was considered to have a better color than the sucrose pack. In the packs containing sucrose plus corn sirup combinations, there was little difference in the color of the various samples.

The best-flavored samples were the (2 + 1) packs, followed by (3 + 1) and (4 + 1) packs. The all-sucrose packs had the best flavor, followed by the 50 per cent sucrose plus 50 per cent corn sirup packs, and the all-corn sirup packs. The flavors of the peaches packed with different concentrations of sirups improved with the increase of total solids in the sirup used.

DISCUSSION

Of the varieties of peaches used in this study, the Gage crossed with Elberta proved to be most satisfactory from the standpoint of flavor, color, and body.

In the selection of a peach to be frozen-packed for later use in ice cream, it was found to be important that the fruit be well ripened, firm yet juicy, of golden yellow color and of distinct flavor. High sugar concentrations did not cause as excessive bleeding of the peach as it does in the case of fruits such as the strawberry. The best flavor usually resulted from the use of a high sugar concentration of two parts fruit and one part of sugar (2 + 1); however, satisfactory results were obtained with (3 + 1) packs. Packs containing only 20 per cent sugar (4 + 1) were not satisfactory because of the loss in flavor during storage.

Dextrose, because of its low solubility, crystallized when used alone. In combination with sucrose or corn sirup, little crystallization occurred in (3 + 1) packs when the proportion of dextrose was not greater than 50 per cent.

Corn sirup produced satisfactory results when used to replace 50 per cent of the sucrose. Such combinations were in some cases superior in flavor to the all-sucrose packs. When the corn sirup was used to replace all the sucrose, the flavor usually was considered less desirable.

Better fruit color resulted from the use of a combination of corn sirup and sucrose than when sucrose was used alone.

In comparing the different methods of adding the sugar and sirup to the fruit, it was found that adding the sugar in dry form gave best results. When sugar and corn sirup both were used, adding the fruit, sugar, and sirup in alternate layers was most satisfactory. Inverting of the containers at least once before final storage was found to aid in uniform mixing of the sugar and sirup with the fruit.

CONCLUSIONS

1. Peaches vary a great deal from the standpoint of their desirability for storage in frozen form.

2. Peaches to be frozen-packed should be well ripened, firm, juicy and of a golden yellow color.

3. For best flavor and color, the proportion of fruit to sweetening agent should be (2 + 1).

4. From the standpoint of flavor and color, a combination of 50 per cent cane sugar and 50 per cent corn sirup proved to be most satisfactory.

5. Because of its low solubility, dextrose should not be used in greater proportions than 50 per cent of the sweetening agent used and should not be used in fruit-sugar ratios in which the proportion of fruit to sugar is less than 3 to 1.

6. When using a combination of sugar and corn sirup, the fruit, sugar, and sirup should be added in alternate layers. There is no particular advantage in combining the sugar and sirup before adding to the fruit.

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HERITABILITY OF HEAT TOLERANCE IN DAIRY CATTLE

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Dairy cattle with a high degree of tolerance to heat are especially desirable in southern regions of the United States. Those possessing this heat-tolerance characteristic would be in special demand if they transmitted portions of it to their offspring. Otherwise, little permanent gain would be made in selecting animals possessing a high tolerance to heat.

Previous studies by Freeborn *et al.* (2) and Seath and Miller (9) have shown that Jerseys appear to be more tolerant to heat than do Holsteins. No attempt was made, however, to measure the degree to which heat tolerance is heritable. The present study was undertaken in an attempt to answer that question. It also was conducted in an effort to determine, if possible, what particular type of observation and how many observations should be made in order to best measure heat tolerance in dairy cattle.

MATERIAL AND METHODS

A description of the cows used and the method of securing the data have been covered in a previous paper (8). In brief, the procedure involved taking body (rectal) temperatures and respiration rates (from flank movements) of milking cows soon after they entered the milking barn at appproximately 3:00 p.m. In 1944, records were taken twice weekly over a period of 13 weeks between July 28 and October 24. During 1945, fifteen observations were made between July 16 and August 24.

The cows were handled in two separate dairy units, thus necessitating observations on separate days. Each unit consisted of both Jersey and Holstein cows. In 1944 there were 36 Holsteins and 16 Jerseys, while in 1945 there were 41 Holsteins and 27 Jerseys. Only 13 Holsteins and 8 Jerseys were the same for the 2 years.

RESULTS

Sire progeny rank and repeatability for two years. Body temperature and respiration rate averages for sire progeny groups (table 1) give some evidence that data taken duing one year are a reasonably good indication of what can be expected another year from groups of cows by the same sire.

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[1] Dr. J. L. Lush of Iowa State College gave much help and many suggestions on how best to conduct this study and aided in analyzing the data secured. Dr. G. E. Dickerson of the Regional Swine Breeding Laboratory, Ames, Iowa, also assisted with statistical analyses. G. D. Miller and Dr. L. L. Rusoff of the Dairy Research Department, Louisiana State University, assisted in gathering the data and gave valuable suggestions in preparation of manuscript.

In 1944 records were averaged on 52 daughters of 7 sires, the number of daughters varying from 4 to 11 per sire. During 1945 the number of daughters by these same 7 sires, plus one new one, totaled 68 and varied from 4 to 17 per sire with only 21 of them being the same as those observed in 1944.

The relative ranks of the respective sire progeny groups are much the same for the 2 years, with body temperature ratings more nearly alike than those for respiration rate. Of special interest are the high ratings on the basis of average body temperature for Holstein groups, with Jersey groups sharing the top ratings on the basis of respiration rate. It will be noted that daughters of Jersey sire no. 7 ranked highest in respiration rate for each of the 2 years, with over 100 respirations per minute. In contrast, the daughters of this same sire ranked sixth for each of the 2 years on the basis of body temperature. In reverse order, the progeny of Holstein sire no. 4 ranked highest in body temperature for both years; yet, on the basis of respiration rate, they ranked sixth in 1944 and seventh in 1945. Likewise, the progeny of sire no. 1 ranked second highest in body temperature each year, yet were seventh and fifth, respectively, in respiration rate for the 2 years.

TABLE 1

Body temperature and respiration rate averages and rank of sire progeny groups
(Progeny of 7 sires, using data on 8 warmer days)*

Sire no.	Breed	Body temperature				Respiration rate per minute			
		Av.		Relative rank		Av.		Relative rank	
		1944	*1945*	*1944*	*1945*	*1944*	*1945*	*1944*	*1945*
1	Holstein	104.27	104.26	2	2	79.0	81.5	7	5
2	"	104.19	103.91	3	3	81.6	86.3	3	4
3	"	103.79	103.89	4	5	88.2	94.2	2	2
4	"	104.40	104.26	1	1	79.2	80.0	6	7
5	Jersey	103.18	103.16	5	7	79.5	80.7	4	6
6	"	102.91	103.90	7	4	79.3	92.9	5	3
7	"	103.09	103.51	6	6	100.2	100.9	1	1

* Air temperature averaged approximately 89° F. for 8 days observed.

Correlation coefficients between the average records for the 8 warm days in 1944 with those for 1945 were computed for the 21 cows common to the study for both years. Results of this study, when considered on an intra-breed-herd basis, yielded *r* values of 0.37 for body temperature and 0.64 for respiration rate. This shows that cows tend to react to warm weather in a given year similarly to the way in which they reacted the previous year.

The use of components of variance in the analysis. Analysis of variance (10) was used to segregate differences (a) between herds (included differences between days on which herds were observed), (b) between breeds within herds, (c) between sires within same breed and herd, (d) between cows within same sire, breed, and herd group, and (e) between records of the same cow. An example of this method of segregating portions of the total variance can be seen in table 2. The procedure used in deriving an estimate of heritability of heat tolerance and the repeatability between single records of the same cow also is shown. This procedure involved, in principle, the computing of intra-class correlations (10)—in one case that between single records of paternal sisters, which was multiplied by 4 in order to secure an estimate of heritability of differences between single records (4), and in the second case that between single records of the same cow, which gave an estimate of average repeatability of single records (5).

TABLE 2

Analysis of variance for body temperature
(Data for milking cows observed on 8 warmer days in 1944)

Source of variance	*d.f.*	Mean square	Composition of mean square
Between herds	1	7.53	
Between breeds within herds	2	45.65	
Between sires within herd and breed	10	2.296	$E + cC + sS$
Between cows within sire, herd, and breed	38	1.43	$E + cC$
Between records of same cow	364	0.688	E

E = Variance between records of same cow = 0.688

c = Number of records per cow = 8

s = Computed number of records per sire group (4) = 28.32

C = Variance between cows within same sire, breed, and herd =

$$\frac{(E + cC) - E}{c} = \frac{1.43 - 0.688}{8} = 0.0927$$

S = Variance between sires within same breed and herd =

$$\frac{(E + cC + sS) - (E + cC)}{s} = \frac{2.296 - 1.43}{28.32} = 0.0306$$

Repeatability between single records of the same cow =

$$\frac{C + S}{E + C + S} = \frac{0.0927 + .0306}{0.688 + 0.0927 + 0.0306} = 0.152$$

Estimate of heritable portion of the variance =

$$\frac{4S}{E + C + S} = \frac{(4)\ 0.0306}{0.688 + 0.0927 + 0.0306} = 0.151$$

Days selected for measuring heat tolerance. A preliminary study was made to determine whether data for all test periods should be used for each year as a measurement of heat tolerance or whether data showing reactions of cows during the warmer days only would give the most accurate information. To determine this answer it was necessary to compare the repeatability of individual cow records when all data were used to that computed using the 8 warmer days, as shown for 1944 in table 2. These comparisons, as given in table 3, show in all cases that body temperature and respiration reactions tended to repeat themselves more closely on the 8 warm days than during the entire test periods. It is probable that this took place because

TABLE 3

Comparison of repeatability of single records of same cow for 8 warmer days versus entire period of test

	Using entire test period		Using 8 warmer days	
	1944	1945	1944	1945
Air temperatures for test periods				
Range	65–93	75–91	86–93	87-91
Average	85	86	89	89
Repeatability of single records				
Body temperature	0.080	0.067	0.152	0.385
Respiration rate	0.254	0.167	0.42	0.478

of the threshold effect, which causes cows to react differently and more as individuals when air temperatures rise above a certain level.

Data for the 8 warmer days also were the most useful in estimating the degree to which heat tolerance is heritable. As with repeatability between single records, it was found that the higher air temperatures as they existed on the 8 warmer days tended to increase the portion of the variance attributable to differences in heredity by from two to four times that found when data for all observation days were considered.

Heritability of body temperature changes. Analysis of the 1944 data for body temperature taken on the 8 warmer days is presented in table 2. Data taken in 1945 have been subjected to the same analysis. Results for the two years are shown in table 4. In general, it appears that the year 1945 produced greater variations that were attributable to differences between cows. As evidence of this, the repeatability between single records of the same cow was 0.385 or 38.5 per cent for 1945 as compared to 15.2 per cent for 1944.

Repeatability and heritability of respiration rate. When the analysis of variance procedure was applied to the respiration data taken on the 8 warmer days, the results (table 5) gave an estimate of repeatability. As shown in table 5, the repeatability in 1945 between records of the same cow was 0.481 or 48.1 per cent. The corresponding value for 1944 was 42 per

TABLE 4

Two-year comparisons of portions of variance concerned with heritability of body temperature

	1944	1945
C = Variance between cows of same sire, breed and herd .	0.0927	0.2030
S = Variance between sires within same breed and herd .	0.0306	0.0508
$\frac{C+S}{E+C+S}$ = Repeatability between single records of same cow	0.152	0.385
$\frac{4S}{E+C+S}$ = Estimate of heritable portion of variance	0.151	0.309

cent. The estimates of heritable portions of variance were surprisingly high, *i.e.*, 84.3 per cent for 1945 and 76.6 for 1944.

TABLE 5

Analysis of variance for respiration rate

(Data for milking cows observed on 8 warmer days in 1945)

Source of variance	*d.f.*	Mean square	Composition of mean square
Between herds	1	123.0	
Between breeds within herds	2	504.5	
Between sires within herd and breed	12	2444.5	$E + cC + sS$
Between cows within sire, herd and breed	52	743.8	$E + cC$
Between records of same cow	473	143.8	E

E = Variance between records of same cow = 143.8

c = Number records per cow = 8

s = Computed number records per sire group = 29.12

C = Variance between cows within same sire, breed, and herd = 75.00

S = Variance between sires within same breed and herd = 58.40

Repeatability between single records of the same cow = 0.481

Estimate of heritable portion of the variance = 0.843

DISCUSSION

Similarity between the ratings in 1944 and 1945 of seven sires on the basis of the response of their daughters to warm weather was quite striking (table 1). These ratings, on the bases of both body temperature and respiration rate. were enough alike for the 2 years to suggest that inheritance must play an important part in causing differences to exist in heat tolerance among dairy cows. Discrepancy between the ratings on the basis of respiration rate as compared to those for body temperature, however, leaves doubt as to the emphasis that should be placed on each of the two heat-tolerance measurements. As was pointed out, the progeny of certain sires ranked near the top in respiration rate, yet near the bottom in body temperature, with the reverse true in one or two cases. This took place even though studies show (3, 8) that both body temperature and respiration rate are correlated closely with air temperature. It would seem that body temperature is probably the safer index on which to judge heat tolerance, since this is no good reason for wanting cows to have high body temperatures. On the other hand, the inheritance of fast breathing by a cow may aid her considerably in quickly eliminating excess heat, which contributes to her comfort (and possibly her health) by more nearly maintaining a normal body temperature.

Repeatability between the averages of warm-weather records for consecutive years among cows of the same breed, based on only 21 cows common to the study for the 2 years, was highest for respiration ($r = 0.64$) and lowest for body temperature ($r = 0.37$). In the case of both respiration and body temperature, these results show that the cow as an individual reacts to warm weather in a manner which is similar from year to year and suggests that the reason for the similarity in reactions must be due, at least partially, to inheritance.

Results of this study are in line with findings by Regan and Freeborn (7) and the general practice of making heat-tolerance observations on warm days only. When 8 warm days were used, the individual records of each cow show repeatability averages for body temperature of 15.2 per cent and 38.5 per cent, respectively, for the 2 years. For respiration rate the repeatability was higher, being 42 per cent and 47.8 per cent, respectively.

Efforts to estimate heritability of individual body temperature records on the basis of sire-progeny differences, although subject to much sampling error, gave results which appear reasonable, *i.e.*, 15.1 per cent in 1944 and 30.9 per cent in 1945. These are approximately the same as the 15.2 per cent and 38.5 per cent which represent the estimates for repeatability of individual records of the same cow for these 2 years. In general, one would expect the repeatability percentage to exceed that for heritability (5), for a cow tends to repeat her performance, not only because of her specific inheritance but also because of certain factors peculiar to herself, including those involved in her environment.

If 15 to 30 per cent is a reasonable estimate of heritability, then one can closely predict what progress can be made through breeding toward more tolerance to high air temperature. For example, if selection in a herd results in saving, as parents, cows (and a bull) that average 1° F. lower in body temperature (when tested on a warm day) than the average of the entire herd, then one would expect offspring from these selected parents to average from 0.15 to 0.3° F. lower than the herd average when subjected to a similar test. This degree of heritability is in line with that found when considering single production records of dairy cows (5, 6). In both cases, as explained by Lush (5), the use of more than one record increases the heritable portion of the variance, although it does lower slightly the spread between the average of those saved and the average for the herd, which is spoken of as the selection differential. Even so, the increase is worthwhile. Using two records with an average heritability of 20 per cent would increase progress from selection by 29 per cent over that secured when only one record is used. The increase would be 58 per cent using four records, and 83 per cent when eight records are used.

Mention already has been made of the unexpected results secured from a study of respiration rates. The estimates for heritability of 76.6 per cent for 1944 and 84.3 per cent for 1945 greatly exceed those for repeatability of single records, which were 42 per cent and 48.1 per cent, respectively, for these 2 years. In general, one can expect repeatability percentages to exceed those for heritability for reasons already explained in connection with body temperature comparisons. Cases where this does not occur can be explained by sampling errors, *e.g.*, calculation of fiducial limits for heritability[2] indicates that results secured are well within the range of expectation, considering number of animals tested and number of sires involved in the estimates.

In general, this experiment has yielded results which indicate that respiration rate as a measure of tolerance to heat, while highly repeatable, gives results which are hard to explain and does not appear to coincide closely enough with body temperature as a measurement to permit general use of respiration rate, to the exclusion of other tests. On the other hand, body temperature, while slightly less repeatable, does appear to be a good test for heat tolerance and yields results which are reasonable.

SUMMARY

Tests of Jerseys and Holsteins involving 52 cows by 7 sires in 1944 and 68 cows by 8 sires in 1945 with respect to the heritability of heat tolerance as indicated by variations in body temperature and respiration rate gave results as follows:

1. Ranking of sire progeny by years showed a great similarity for the 2 years, although there was discrepancy between rank on basis of body temperature and that for respiration rate. Some sire groups ranked high on one basis and low on the other and vice versa.
2. Twenty-one cows included in study for both years showed correlations between average records for 8 warmer days (on an intra-herd-breed basis) of 0.37 for body temperature and 0.64 for respiration rate.
3. Using records for 8 warmer days gave a repeatability for individual body temperature records of same cow of 15.2 per cent for 1944 and 38.5 per cent for 1945, as compared to 8 per cent and 6.7 per cent for the 2 years when all observation days were used. In like manner, respiration rates were more highly repeatable using only the warmer days.
4. Estimates of heritability of individual records based on sire-progeny differences were for body temperature 15.1 per cent and 30.9 per cent for the 2 years, and for respiration 76.6 per cent and 84.3 per cent. Figures for respiration appear out of line, as they greatly exceed the estimates of repeatability and the reverse condition was expected.
5. Body temperature appears to be a safer measuring stick for heat tolerance than does respiration rate.
6. The estimate of heritability of body temperature (15 to 30 per cent) is in line with that found for individual production records of cows. In practice this would mean that the offspring from parents selected because of their tolerance to heat would be expected to retain from 15 to 30 per cent of the advantage that the parents had over the average for the herd or breed.

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[2] Fiducial limits at the 5 per cent level of significance computed as per methods outlined by Fisher (1) resulted in estimates of heritability of single respiration records ranging from 22 to 177 per cent in 1944 and from 25.6 to 232 per cent in 1945.

7. Using more than one record greatly increases progress through selection. If heritable portion of variance between single heat tolerance record is 20 per cent, then progress through selection would increase by 29 per cent using two records, 58 per cent with four, and 83 per cent using eight records.

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A COMPARISON OF THE BABCOCK, GERBER, MINNESOTA, PENNSYLVANIA, AND MOJONNIER METHODS FOR DETERMINING THE PERCENTAGE OF FAT IN HOMOGENIZED MILK[1]

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The general acceptance of homogenized milk today has made imperative a study of the existing methods for testing it for butterfat. Extensive studies have been made by several workers on the various methods of testing nonhomogenized milk for butterfat, but the data are somewhat limited when the methods are applied to homogenized milk.

LITERATURE

The Roese-Gottlieb (Mojonnier) Method

Burr (6), comparing several methods for testing homogenized milk for fat, found the Roese-Gottlieb method the most accurate. This test was so considered by all the chemists at that time. Richmond (28) pointed out that for ease and accuracy the Roese-Gottlieb method appeared to be the best method for determining the percentage of fat in homogenized milk. Marquardt (23) stated that ether extraction methods gave the most reliable results when testing homogenized milk for fat. Doan (12) stated that the homogenization process did not influence the accuracy of the Roese-Gottlieb or the Mojonnier methods. Mojonnier (25) reported the results of nine tests made by the Kohler and Kohler Laboratories showing that homogenized milk tested by the Mojonnier method averaged 0.012 per cent less than the same milk not homogenized. Dahlberg, Holm, and Troy (11), in comparing the Roese-Gottlieb tests of milk made by five different laboratories, double homogenized one sample at 2,500 lbs. pressure to render it homogeneous for all laboratories. The sample of homogenized milk did not yield tests with the least variation between duplicates or between laboratories.

The Babcock Method

The literature on the Babcock method for testing homogenized milk has been reviewed by Herreid (15) and by Trout and Lucas (34) and need not be repeated in full here. However, some review seems necessary to point out the relationship of the procedure in question with the Mojonnier method.

Nonhomogenized milk. Dahlberg (9) showed, in a comparison of 32 tests of nonhomogenized milk varying in fat content from 4.42 to 4.92 per cent, the Babcock test to be an average of 0.1 per cent high, reading from

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the bottom of the lower meniscus to the extreme top of the upper meniscus. Phillips (27) on 50 trials observed that the Babcock tests of nonhomogenized milk ranged from 0.005 to 0.126 and averaged 0.0588 per cent higher than the Roese-Gottlieb tests. Wilster and Robichaux (36) found in testing 1,380 samples that the Babcock test of nonhomogenized milk ranged from 0.074 to 0.077 per cent higher than the Mojonnier tests. They reported no studies on homogenized milk samples.

Hileman *et al.* (18), surveying the literature, reported that twelve of fourteen investigators secured results with the Babcock test on nonhomogenized milk slightly higher than the Mojonnier, the over-all average being + 0.076 per cent above that of the Mojonnier. Fahl, Lucas, and Baten (13) found the Babcock tests higher than those for the Mojonnier. Herreid *et al.* (16), in accounting for milk fat, secured results by the Mojonnier method on unpreserved milk which agreed with those obtained by the Babcock method.

On the other hand, some investigators reported lower tests by the Babcock method than by the Mojonnier. Dahlberg (9), although securing higher results by the Babcock method when the fat column was read according to standard technic, nevertheless did secure lower tests when oil was applied to destroy the meniscus. Dahlberg, Holm, and Troy (11) did not verify the results of several previous investigations which tended to show that the Babcock test gave slightly but uniformly higher percentages of fat than the Mojonnier.

Mojonnier and Troy (24) reported data showing that the difference between the Babcock and Mojonnier tests was not constant in one direction but that the Babcock test varied both above and below that of the Mojonnier. Later, Mojonnier (25) reported the results of Babcock tests on nine samples of homogenized milk which averaged 0.072 per cent higher than the Mojonnier tests. Other tests indicated a higher percentage of fat in the fat columns of test of homogenized milk than in those on nonhomogenized milk.

From calculations of the data presented by Babcock (3) in introducing his test, of 30 samples of whole milk tested by the Babcock and gravimetric methods, 15 Babcock tests were above and 15 were below those of the ether-extraction method. The Babcock test results varied from − 0.16 to + 0.30 per cent from results of the gravimetric procedure. The average tests differed by 0.01 per cent, the Babcock method being higher.

Homogenized milk. Generally the Babcock test of homogenized milk yields results slightly lower than on the same milk not homogenized, being within the 0.1 per cent tolerance for the test. Workers of the Arizona Experiment Station (1) reported comparison of some 30 samples of homogenized milk from different sources, and homogenized at different pressures, indicated that the Mojonnier test was from 11 to 25 per cent higher than the Babcock test. Later (2) they reported data on six trials of milk homogenized from 2,000 to 4,000 lbs. pressure which showed that the Mojonnier test consistently gave results higher than the Babcock and that the difference was believed sufficient to justify a correction when the Babcock method was used for homogenized milk.

Doan (12), using a modified Babcock method, secured results from 0.084 to 0.11 per cent higher than those by the Mojonnier method, the differences being only insignificantly higher than the average in the literature for nonhomogenized milk. Lampert and Brandon (21) secured results on homogenized milk within an average of + 0.03 per cent of the Mojonnier results when using the regular Babcock method on unpreserved samples. This was within 0.01 per cent of the Babcock tests on nonhomogenized milk. Homogenized samples preserved with formalin averaged 0.13 per cent less than by the extraction method. They concluded that the regular Babcock procedure gave accurate results on unpreserved homogenized milk, but a lower test when the sample was preserved with formaldehyde. Trout and Lucas (34) reported that many modifications of the Babcock method existed. Averages of results of five trials on each of eleven modified Babcock tests varied from those of the Mojonnier test from −0.03 to −0.15 per cent, depending chiefly upon the modification.

Webster (35) observed in four trials on homogenized milk that the Babcock test averaged 0.088 per cent lower than the Mojonnier test. He reported that the variation between the Babcock and Mojonnier methods was affected directly by the size of the fat globules or, more particularly, by the relative number of small fat globules present. He concluded that the statement that the Babcock test over-reads the Mojonnier or gravimetric method by some stated percentage seems of little value unless the size of the fat globules is considered.

The Gerber Method

Nonhomogenized milk. Fisher and Walts (14) found the average variation from the Roese-Gottlieb was ± 0.137 per cent for the Babcock and ± 0.122 per cent for the Gerber method in testing nonhomogenized milk. Both the Babcock and the Gerber tests for milk in 11 instances (68.75 per cent) gave results which were slightly higher than the Roese-Gottlieb. They believed there was no advantage in introducing to the industry another method, the Gerber, which was not more accurate than the Babcock method. Dahlberg, Holm, and Troy (11) and Dahlberg (10), applying the test to nonhomogenized milk, found that the Gerber and Babcock tests were comparable from the standpoint of accuracy but recommended that one good practical test for fat in milk was better than two of equal merit.

Van der Burg (5) compared the Gerber with other fat tests, including the Roese-Gottlieb, on 85 samples of milk. Generally the Gerber gave slightly higher results than the other methods. In 12 samples the Gerber fat values were identical with other values, in 31 samples they were 0.005

to 0.025 per cent higher, and in 24 samples 0.005 to 0.025 per cent lower. Fourteen samples varied ± 0.030 to ± 0.050 per cent and 4 samples ± 0.055 to ± 0.100 per cent.

Homogenized milk. Buttenberg (8) pointed out that the determination of the fat content of homogenized milk by the Adams method, commonly used at that time, could not show results comparable with the Roese-Gottlieb method; even when the extraction was prolonged purposely from 10 to 12 hours, the results were much too low.

Siegfeld (29) used the Gerber method for testing homogenized milk with excellent results, but pointed out that it was necessary in testing homogenized milk by this method to centrifuge 12 minutes instead of the customary 3 minutes. Burr (6), comparing several methods, found that the Gerber method, while fairly satisfactory, gave results which varied with the time of centrifuging. The homogenized milk examined was processed at 60 atmospheres (approximately 900 lbs.) pressure. Average results of 11 trials showed the Gerber test centrifuged 10 minutes varied from the Roese-Gottlieb by ± 0.02 per cent, - 0.08 to + 0.125 per cent being the range of variation.

Richmond (28) found the Gerber test gave good results with homogenized milk, but that "the advent of homogenized milk rendered it necessary to remove the Adams method from the position it had so long occupied as a standard method." Six trials showed that the Gerber test varied ± 0.008 per cent from the Roese-Gottlieb tests of the same milk. Istaz and Van Soest (20) observed that the results secured on homogenized milk by the Gerber method were verified by the gravimetric method in many cases.

Hoyberg (19) had difficulty securing results by the Gerber method comparable with those of the Roese-Gottlieb method, even when centrifuging as long as 45 to 60 minutes, or when prolonging the holding time in the water bath after centrifuging. By heating the milk to 60–65° C., holding it 5 minutes and then making the Gerber test, results identical with those obtained by the Roese-Gottlieb method were secured. He advised pouring heated milk directly into the sulfuric acid and amyl alcohol mixture rather than letting it run down the side of the butyrometer. This resulted in an increased amount of heat liberation, which was deemed important in testing homogenized milk. Milk heated to 15° C. increased to 75° C. during its reaction with the sulfuric acid and amyl alcohol. At 25° C. it increased to 82°, at 40° to 86°, and at 45° to 88° C. Milk heated to 60–65° C. would reach a temperature of 105° C. when added to the acid.

Burr and Weise (7) found that the Gerber method gave comparable but slightly higher fat tests of homogenized milk than the Roese-Gottlieb method. However, in using the Gerber test, double centrifuging was necessary. In 18 trials the Gerber method gave results 0.03 per cent higher than the Roese-Gottlieb method. Von Sobbe (30) found the Gerber method very satisfactory for testing homogenized milk, but noted that centrifuging had to be repeated at least twice, and that the mixture of sulfuric acid and milk plasma had to be clear and transparent. In making the test he warmed the milk to 60–65° C., then cooled it with agitation to remove the disturbing effects of homogenization. Herrington (17) pointed out that the amyl alcohol used in the Gerber test was a possible source of error in the test. The error caused by the test might be 0.1–0.2 per cent or even higher. He recommended that boiling between 128° C. and 132° C. was the best method of identifying any sample of amyl alcohol to be used in making the Gerber test.

The Minnesota-Babcock Method

While the Minnesota-Babcock test is used for the fat determination of all dairy products (26), few data were found in the literature on its use in testing homogenized milk. Trout, Halloran, and Gould (33) and Trout (32) studied the possibilities of the Minnesota reagent for overcoming char formation in testing homogenized milk. They reported results comparable in appearance with the best results obtained by the Babcock method but presented no data comparing the accuracy of the two methods.

Lampert and Brandon (21) on two trials secured tests on homogenized milk by the Minnesota method which ranged from 0.22 to 0.26 per cent less than the Mojonnier tests. The samples were preserved with mercuric chloride. They stated, "Samples preserved with mercuric chloride reacted with the reagent so that a black precipitate, probably finely divided mercury, was formed. This material entered the fat column, but in a number of cases the fat column dropped through this material, leaving it adhering to the neck of the test bottle. Although the fat tests appeared excellent, the results were lower than those obtained with the other procedures (Mojonnier, Babcock, and Pennsylvania), and the use of the Minnesota test was not continued." They concluded that the method could not be recommended for the testing of homogenized milk. Bird and Breazeale (4) observed wide variations in the fat content of the same sample of buttermilk tested by three Minnesota reagents.

The Pennsylvania Method

Few data were found on the use of the Pennsylvania method for testing homogenized milk. Swope (31) recommended it for the testing of homogenized milk. He reported several tests on two samples of homogenized milk, one sample of which showed an average arithmetical deviation from the Mojonnier test of 0.036 per cent and an average algebraic deviation + 0.024 per cent. The second sample showed an arithmetical deviation of 0.047 per cent and an average algebraic deviation of + 0.041 per cent. The range in deviation of the Pennsylvania tests from the Mojonnier tests was from − 0.009 to + 0.091 per cent.

Lampert and Brandon (21) found the Pennsylvania test yielded an average of 0.10 per cent less fat on the homogenized milk than on the same milk not homogenized, the tests of the nonhomogenized and homogenized milk being +0.22 and +0.17 per cent higher, respectively, than those obtained by the Mojonnier method. Difficulty was encountered in making satisfactory tests of homogenized milk to which preservatives had been added. They concluded that the Pennsylvania test could not be recommended for the testing of homogenized milk.

SCOPE OF THE INVESTIGATION

Inasmuch as homogenized milk is of increasing importance in market milk distribution, a comparative study of the various more common methods of testing milk for fat seemed advisable. In this investigation the following methods were compared: Mojonnier, modified Babcock, Gerber, Minnesota-Babcock, Pennsylvania, and a modified Pennsylvania method. Data were secured from duplicate tests on 24 samples for each of the above methods. These tests were not necessarily designed for testing homogenized milk. However, it seemed desirable to include all of them in the study since some of them (a) are more or less common tests in testing laboratories, (b) are used in testing milk in vocational high schools, (c) employ chemicals other than sulfuric acid, which might thus prevent char formation, (d) are readily available, (e) are moderately priced, and (f) are not too difficult for routine analyses.

PROCEDURE

The milk tested was that regularly processed in the College Creamery. The nonhomogenized samples were taken from the vat after pasteurization and prior to homogenization. The milk had been kept thoroughly mixed during pasteurization and homogenization in order to insure uniform fat distribution. The homogenized samples were taken from the cooled bottled product after homogenization was well under way. Mojonnier tests showed that the nonhomogenized and homogenized samples contained similar percentages of fat.

Homogenization was done by means of a 500-gallon-per-hour viscolizer at 2,500 lbs. pressure at 130° to 140° F. following pasteurization. The collected samples were cooled adequately, stored and tested as rapidly as time would permit. Pipetting of the portions of milk into the test bottles was done for all tests at one time to assure correct sampling. This was done after the milk had been tempered at 70° F. for 2 hours. These charged test bottles were then stored at 40° F. until the tests were made.

The Mojonnier test was used as a standard for accuracy. Instead of using an approximately ten-gram portion measured volumetrically, duplicate samples of milk previously tempered at 70° F. were weighed carefully on a chemical balance directly into a fat-extraction flask.

The tests using Babcock test bottles were made in bottles which had been recalibrated for accuracy. Any bottles showing 0.1 per cent or more variation from exact accuracy were discarded. The modified Babcock procedure employed 17.5 ml. of 1.835 specific gravity sulfuric acid added in three portions, 8.0, 5.0 and 4.5 ml., respectively. Mixing was prolonged for at least 2 minutes after final addition of the acid, as suggested by Lucas and Trout (22); however, the water-alcohol solution was not added to support the fat column for reading. The Gerber (Fucoma) test was carried out according to the directions of the Fucoma Company. The reagents used in the Minnesota-Babcock method were from the Kimble Glass Co. The modification of the Pennsylvania test consisted in the use of sulfuric acid having a specific gravity of 1.81 instead of 1.73, as recommended and as used in the regular procedure.

RESULTS

The Mojonnier method. The data secured on testing nonhomogenized and homogenized milk by the Mojonnier method are presented in table 1.

TABLE 1

Mojonnier fat tests of nonhomogenized and homogenized milk

Series*	Mojonnier method						
	Nonhomogenized milk			Homogenized milk			Variation from test of nonhomogenized milk
	Duplicates		Av.	Duplicates		Av.	
	%	%		%	%		
1	3.651	3.650	3.65	3.672	3.671	3.67	+ 0.02
2	3.708	3.699	3.70	3.701	3.694	3.70	0.00
3	3.695	3.716	3.71	3.712	3.695	3.70	− 0.01
4	3.723	3.716	3.72	3.724	3.738	3.73	+ 0.01
5	3.814	3.801	3.81	3.826	3.817	3.82	+ 0.01
6	3.831	3.804	3.82	3.804	3.815	3.81	− 0.01
7	3.850	3.864	3.86	3.835	3.832	3.83	− 0.03
8	3.916	3.886	3.90	3.898	3.882	3.89	− 0.01
9	3.897	3.926	3.91	3.918	3.903	3.91	0.00
10	4.375	4.409	4.39	4.407	4.404	4.41	+ 0.02
11	4.470	4.476	4.47	4.493	4.503	4.50	+ 0.03
12	4.532	4.537	4.53	4.543	4.552	4.55	+ 0.02
13	4.585	4.575	4.58	4.560	4.570	4.57	− 0.01
14	4.556	4.592	4.57	4.594	4.593	4.59	+ 0.02
15	4.591	4.589	4.59	4.580	4.600	4.59	0.00
16	4.591	4.596	4.59	4.597	4.586	4.59	0.00
17	4.575	4.628	4.60	4.653	4.634	4.64	+ 0.04
18	4.619	4.606	4.61	4.608	4.606	4.61	0.00
19	4.606	4.627	4.62	4.641	4.644	4.64	+ 0.02
20	4.763	4.761	4.76	4.759	4.768	4.76	0.00
21	4.786	4.789	4.79	4.785	4.777	4.78	− 0.01
22	4.956	4.973	4.96	4.936	4.934	4.94	− 0.02
23	5.012	5.038	5.03	5.049	5.04	5.04	+ 0.01
24	5.124	5.082	5.10	5.04	5.08	5.06	− 0.04
Av.			4.344			4.347	0.0024

Arranged according to increasing percentages of fat.

The average test was 4.344 per cent for nonhomogenized milk and 4.347 per cent for homogenized milk. Of the 24 samples, 6 tested exactly the same as the nonhomogenized; 8 tested lower, ranging from −0.01 to −0.04 per cent; and 10 tested higher, ranging from +0.01 to +0.04 per cent. Thus it appears that homogenized milk may be tested reliably by the Mojonnier method.

The modified Babcock method. The same samples of milk tested by the Mojonnier method also were tested by the modified Babcock method. The nonhomogenized and homogenized milk averaged 4.402 and 4.390 per cent butterfat, respectively (table 2). Of the 24 samples tested, 10 tests were identical with those of the nonhomogenized milk; 12 were lower, ranging from −0.02 to −0.07; and 2 were higher, +0.02 and +0.05. The nonhomogenized milk averaged 0.057 per cent higher by this method than by the Mojonnier; the homogenized averaged only 0.043 per cent higher.

TABLE 2

Comparison of modified Babcock and Mojonnier tests of homogenized milk

Series*	Modified Babcock method			Variations from Mojonnier	
	Nonhomogenized	Homogenized	Variation of homogenized from nonhomogenized	Nonhomogenized	Homogenized
	%	%			
1	3.72	3.70	−0.02	+0.07	+0.03
2	3.72	3.70	−0.02	+0.02	0.00
3	3.77	3.75	−0.02	+0.06	+0.05
4	3.78	3.78	0.00	+0.06	+0.05
5	3.90	3.88	−0.02	+0.09	+0.06
6	3.85	3.85	0.00	+0.03	+0.04
7	3.90	3.88	−0.02	+0.04	+0.05
8	3.98	3.93	−0.05	+0.08	+0.04
9	3.95	3.93	−0.02	+0.04	+0.02
10	4.48	4.48	0.00	+0.09	+0.07
11	4.53	4.53	0.00	+0.06	+0.03
12	4.58	4.58	0.00	+0.05	+0.03
13	4.63	4.63	0.00	+0.05	+0.06
14	4.67	4.60	−0.07	+0.10	+0.01
15	4.65	4.63	−0.02	+0.06	+0.04
16	4.63	4.63	0.00	+0.04	+0.04
17	4.67	4.65	−0.02	+0.07	+0.01
18	4.70	4.70	0.00	+0.09	+0.09
19	4.68	4.70	+0.02	+0.06	+0.06
20	4.80	4.80	0.00	+0.04	+0.04
21	4.80	4.80	0.00	+0.01	+0.02
22	5.00	4.95	−0.05	+0.04	+0.01
23	5.08	5.13	+0.05	+0.05	+0.09
24	5.17	5.15	−0.02	+0.07	+0.09
Av.	4.402	4.390	−0.012	+0.057†	+0.043†

* The tests reported in this table were included among the data on the 36 trials previously reported by Lucas and Trout (22).

† Highly significantly different from zero.

The Gerber method. The average Gerber tests of the nonhomogenized and homogenized milk were practically identical, the average test on homogenized milk being 0.003 per cent higher (table 3). Of the 24 comparisons, 12 were the same; 5 of the homogenized were lower, ranging from −0.02 to −0.07 per cent; and 7 were higher, ranging from +0.02 to +0.08 per cent. The average tests of both the nonhomogenized and the homogenized averaged 0.09 per cent higher than the corresponding Mojonnier tests. In making the Gerber tests of homogenized milk the following factors were striking: (a) the clarity of the fat column and supporting liquid, (b) the identical reading of the duplicate tests, (c) the consistent check with tests on the nonhomogenized milk, and (d) the complete freedom of any char formation.

TABLE 3

Comparison of Gerber and Mojonnier tests of homogenized milk

Series	Gerber method			Variations from Mojonnier	
	Nonhomogenized	Homogenized	Variation of homogenized from nonhomogenized	Nonhomogenized	Homogenized
	%	%			
1	3.73	3.73	0.00	+0.08	+0.06
2	3.78	3.78	0.00	+0.08	+0.08
3	3.80	3.80	0.00	+0.09	+0.10
4	3.83	3.83	0.00	+0.11	+0.10
5	3.93	3.95	+0.02	+0.12	+0.13
6	3.85	3.87	+0.02	+0.03	+0.06
7	3.95	3.95	0.00	+0.09	+0.12
8	4.00	4.00	0.00	+0.10	+0.11
9	4.03	3.96	−0.07	+0.12	+0.05
10	4.50	4.52	+0.02	+0.11	+0.11
11	4.60	4.58	−0.02	+0.13	+0.08
12	4.60	4.62	+0.02	+0.07	+0.07
13	4.68	4.68	0.00	+0.10	+0.11
14	4.72	4.70	0.00	+0.15	+0.11
15	4.65	4.67	+0.02	+0.06	+0.08
16	4.70	4.70	0.00	+0.11	+0.11
17	4.68	4.73	+0.05	+0.08	+0.09
18	4.70	4.68	−0.02	+0.09	+0.07
19	4.67	4.75	+0.08	+0.05	+0.11
20	4.85	4.83	−0.02	+0.09	+0.07
21	4.88	4.88	0.00	+0.09	+0.10
22	5.00	4.98	−0.02	+0.04	+0.04
23	5.10	5.10	0.00	+0.07	+0.06
24	5.20	5.20	0.00	+0.10	+0.14
Av.	4.434	4.437	+0.003	+0.090*	+0.090*

* Highly significantly different from zero.

The Minnesota method. While the Minnesota-Babcock test of homogenized milk varied from that of the nonhomogenized milk by an average of only +0.027 per cent, the range of variations between the tests extended from −0.32 to +0.40 per cent. Only 2 of the 24 tests were identical with those of the nonhomogenized milk, while 12 were below, ranging from −0.02 to −0.30, and 10 were above, ranging from +0.07 to +0.40 per cent (table 4). Tests of both nonhomogenized and homogenized milk were consistently under the Mojonnier tests, the tests on the homogenized milk ranging from −0.04 to −0.85 and averaging −0.433 per cent.

The Pennsylvania method. The Pennsylvania tests on homogenized milk consistently were under those of the nonhomogenized milk, −0.07 to −0.72 and averaging −0.538, and were under the Mojonnier readings in 18 of the 24 trials, or 75 per cent (table 5). The readings ranged from −0.31 to −0.50 per cent under those of the Mojonnier and from +0.03 to +0.16 per cent above, and averaged −0.29 per cent. Nevertheless, the same tests on the nonhomogenized milk consistently were above those of the Mojonnier, averaging +0.255 per cent higher.

TABLE 4

Comparison of Minnesota and Mojonnier tests of homogenized milk

Series	Minnesota method			Variations from Mojonnier	
	Nonhomogenized	Homogenized	Variation of homogenized from nonhomogenized	Nonhomogenized	Homogenized
	%	%			
1	3.07	3.35	+0.28	−0.58	−0.32
2	3.08	2.88	−0.20	−0.62	−0.82
3	3.05	3.35	+0.30	−0.66	−0.35
4	3.38	3.28	−0.10	−0.34	−0.45
5	3.25	3.23	−0.02	−0.56	−0.59
6	3.50	3.43	−0.07	−0.32	−0.38
7	3.15	2.98	−0.17	−0.71	−0.85
8	3.22	3.20	−0.02	−0.68	−0.69
9	3.33	3.48	+0.15	−0.58	−0.43
10	4.00	4.07	+0.07	−0.39	−0.34
11	4.23	3.93	−0.30	−0.24	−0.57
12	4.00	4.30	+0.30	−0.53	−0.25
13	4.08	4.40	+0.32	−0.50	−0.17
14	3.95	3.95	0.00	−0.62	−0.64
15	4.30	4.55	+0.25	−0.29	−0.04
16	4.23	4.35	+0.12	−0.36	−0.24
17	4.47	4.15	−0.32	−0.13	−0.49
18	4.05	4.20	+0.15	−0.55	−0.41
19	4.20	3.98	−0.22	−0.42	−0.66
20	4.57	4.45	−0.12	−0.19	−0.31
21	4.20	4.60	+0.40	−0.59	−0.18
22	4.43	4.35	−0.08	−0.53	−0.59
23	4.76	4.76	0.00	−0.27	−0.28
24	4.77	4.70	−0.07	−0.33	−0.36
Av.	3.886	3.913	+0.027	−0.458*	−0.433*

* Highly significantly different from zero.

a. *The modified Pennsylvania method.* Since the average Pennsylvania tests on the homogenized milk were lower than both the Pennsylvania test and the Mojonnier test for nonhomogenized milk, attempts were made to improve the test for homogenized milk by increasing the specific gravity of the acid used from 1.73 to 1.81. This modification increased the reading on the nonhomogenized milk slightly, averaging about 0.02 per cent, and that of the homogenized milk about 0.53 per cent, bringing the average readings of the nonhomogenized and homogenized milk within 0.032 per cent of each other (table 6). The tests on the homogenized milk became consistently higher than those of the Mojonnier, averaging + 0.238, whereas the regular Pennsylvania tests were under those of the Mojonnier in 75 per cent of the cases. The wide variation encountered between the tests of nonhomogenized and homogenized milk when made by the Pennsylvania method were narrowed appreciably when sulfuric acid of 1.81 specific gravity was used in the test (table 6 and fig. 1). Nevertheless, the average readings were considerably above those of the Mojonnier (fig. 1).

TABLE 5

Comparison of Pennsylvania and Mojonnier tests of homogenized milk

Series	Pennsylvania method			Variations from Mojonnier	
	Nonhomogenized	Homogenized	Variation of homogenized from nonhomogenized	Nonhomogenized	Homogenized
	%	%			
1	3.88	3.28	− 0.60	+ 0.23	− 0.39
2	3.87	3.20	− 0.67	+ 0.17	− 0.50
3	4.05	3.83	− 0.22	+ 0.34	+ 0.13
4	4.05	3.88	− 0.17	+ 0.33	+ 0.15
5	4.15	3.85	− 0.30	+ 0.34	+ 0.03
6	4.03	3.38	− 0.65	+ 0.21	− 0.43
7	4.20	3.88	− 0.32	+ 0.34	+ 0.05
8	4.20	4.00	− 0.20	+ 0.30	+ 0.11
9	4.17	3.55	− 0.62	+ 0.26	− 0.36
10	4.65	3.98	− 0.67	+ 0.26	− 0.43
11	4.67	4.10	− 0.57	+ 0.20	− 0.40
12	4.80	4.08	− 0.72	+ 0.27	− 0.47
13	4.82	4.10	− 0.72	+ 0.24	− 0.47
14	4.82	4.20	− 0.62	+ 0.25	− 0.39
15	4.82	4.75	− 0.07	+ 0.23	+ 0.16
16	4.85	4.15	− 0.70	+ 0.26	− 0.44
17	4.90	4.30	− 0.60	+ 0.30	− 0.34
18	4.80	4.15	− 0.65	+ 0.19	− 0.46
19	4.85	4.18	− 0.67	+ 0.23	− 0.46
20	5.00	4.30	− 0.70	+ 0.24	− 0.46
21	5.00	4.43	− 0.57	+ 0.21	− 0.45
22	5.22	4.50	− 0.72	+ 0.26	− 0.44
23	5.27	4.65	− 0.62	+ 0.24	− 0.39
24	5.32	4.75	− 0.57	+ 0.22	− 0.31
Av.	4.599	4.061	− 0.538	+ 0.255*	− 0.290*

* Highly significantly different from zero.

The data for all tests are summarized in table 7 and figure 1.

TABLE 6

Comparison of modified Pennsylvania and Mojonnier tests on homogenized milk

Series	Modified Pennsylvania method			Variations from Mojonnier	
	Nonhomogenized	Homogenized	Variation of homogenized from nonhomogenized	Nonhomogenized	Homogenized
	%	%			
1	3.90	3.88	−0.02	+0.25	+0.21
2	3.95	3.90	−0.05	+0.25	+0.20
3	4.05	4.00	−0.05	+0.34	+0.30
4	4.08	4.08	0.00	+0.36	+0.35
5	4.15	4.13	−0.02	+0.34	+0.31
6	4.02	4.00	−0.02	+0.20	+0.19
7	4.15	4.15	0.00	+0.29	+0.32
8	4.22	4.20	−0.02	+0.32	+0.31
9	4.20	4.15	−0.05	+0.31	+0.24
10	4.63	4.55	−0.08	+0.24	+0.14
11	4.78	4.68	−0.10	+0.31	+0.18
12	4.77	4.75	−0.02	+0.24	+0.20
13	4.78	4.80	+0.02	+0.20	+0.23
14	4.85	4.87	+0.02	+0.28	+0.28
15	4.87	4.85	−0.02	+0.28	+0.26
16	4.83	4.83	0.00	+0.24	+0.24
17	4.90	4.80	−0.10	+0.30	+0.16
18	4.87	4.82	−0.05	+0.26	+0.21
19	4.90	4.88	−0.02	+0.28	+0.24
20	5.03	4.93	−0.10	+0.27	+0.17
21	5.03	5.03	0.00	+0.24	+0.25
22	5.23	5.20	−0.03	+0.27	+0.26
23	5.30	5.23	−0.07	+0.27	+0.19
24	5.35	5.35	0.00	+0.25	+0.29
Av.	4.618	4.585	−0.032	+0.274*	+0.238*

* Highly significantly different from zero.

TABLE 7

Comparison of various fat tests of nonhomogenized and homogenized milk (Av. 24 trials)

Test	Percentage fat in		Variation of test of homogenized milk from that of nonhomogenized milk	Variation of test of homogenized milk from Mojonnier test
	Nonhomogenized milk	Homogenized milk		
Mojonnier	4.344	4.347	+0.003	
Babcock*	4.402	4.390	−0.012	+0.043†
Gerber	4.434	4.437	+0.003	+0.090†
Minnesota	3.886	3.913	+0.027	−0.433†
Pennsylvania	4.599	4.061	−0.538	−0.290†
Pennsylvania‡	4.618	4.585	−0.032	+0.238†

* Acid and milk at 70° F., 17.5 ml. of 1.835 sp. gr. acid added in 3 portions, shaken by hand after each addition and finally shaken in shaking machine for at least 2 minutes.

† Highly significantly different from zero.

‡ Specific gravity of acid adjusted to 1.81 instead of the recommended specific gravity of 1.73.

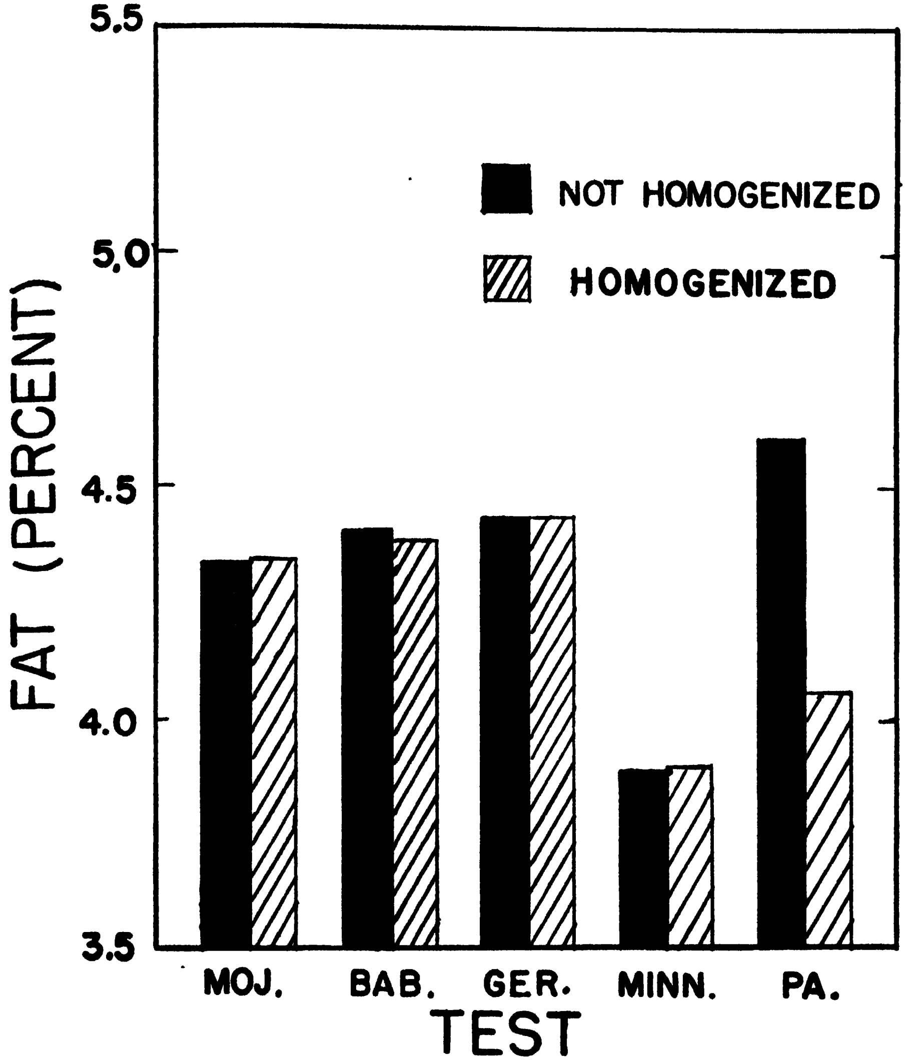

FIG. 1. Fat percentage in milk as determined by the Mojonnier, modified Babcock, Gerber, Minnesota, and Pennsylvania method (average 24 trials).

CONCLUSIONS

Homogenization does not affect the Mojonnier fat test of milk.

The modified Babcock method (17.5 ml. of 1.835 sp. gr. sulfuric acid added in three portions, 8, 5, and 4.5 ml., respectively, and shaken for at least 2 minutes before centrifuging) may be used with much assurance of accuracy in testing homogenized milk. Twenty-four tests in duplicate averaged within – 0.012 per cent of those of nonhomogenized milk made by the same method and within + 0.043 per cent of the Mojonnier average.

Homogenization does not affect the Gerber test. The average Gerber tests, both of nonhomogenized and homogenized milk, were found to be 0.09 per cent higher than those secured by the Mojonnier method. Aside from the necessity of introducing another test and the fact that the readings were approximately 0.1 per cent higher than the Mojonnier, the Gerber test was by all odds the most satisfactory test studied for making fat tests of homogenized milk.

While the Minnesota method yielded average tests of homogenized milk within +0.027 per cent of those of nonhomogenized milk, the tests varied from those of the Mojonnier on the average by − 0.433 per cent. It would seem, therefore, that the test could not be recommended for testing homogenized milk.

The Pennsylvania method, yielding tests on homogenized milk in these studies markedly lower than the Mojonnier method, cannot be recommended for testing homogenized milk.

Credit is due Mr. Robert Frantz for the making of the tests and Dr. W. D. Baten for the testing of significance of some of the data reported herein.

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A METHOD FOR MEASURING THE BODY OF CULTURED CREAM

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The body or viscosity of cultured cream has been measured by various investigators. After uniform agitation of the cultured cream, Guthrie (2) used a MacMichael viscosimeter to determine differences in the body of the product. Two methods were employed by Doan and Dahle (1), namely: (*a*) a viscosimeter of the rotating type and (*b*) "a special plunger test where the penetration of a suitable plunger falling a unit distance into the product was measured". No details were given as to the plunger or method of testing. Joffe (3) describes a method of testing the consistency of mayonnaise and salad dressing, two products which have a consistency similar to cultured cream. He used an instrument called a "plumit". This is a graduated rod of aluminum, bearing a pointed and weighted head of larger diameter than the rod. The total weight is 14.5 g. and the over-all length 13 cm. The plumit is held between the thumb and forefinger and dropped into a jar of mayonnaise from a height of 12 inches. When the plumit is released and drops into the sample, it should remain perpendicular, and a reading of the depth of penetration is taken at once.

Body structure or the apparent viscosity of cultured cream, developed without the addition of stabilizing agents, may be reduced readily to a basic viscosity by means of gentle agitation. It seems desirable to evaluate the uniformity of the product as sold to the consumer by measuring the body of the cultured cream while in the final container and without reducing the apparent viscosity. An aluminum rod, drilled, tapered, and graduated, has been constructed for this purpose. The size of the hole (13/32″ × 3 3/4″) was selected to provide a desirable ratio of weight to volume displacement. The dimensions and mode of construction of this device, called a plummet, are given in figure 1. A release for the plummet (made from a glass tube and a board), a stop watch with a second hand, and a ring stand and clamp also are needed.

The procedure for conducting the test is as follows:

(a) Hold samples to be tested overnight at 40° F. If the retail container is less than 4 inches high and the exposed surface less than 2 inches in diameter, suitable containers (such as an 8-oz. mayonnaise jar) should be employed and filled and handled in a manner identical to the commercial product. Avoid agitating the product prior to testing.

(b) Firmly secure the glass tube in a vertical position with a suitable

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clamp, having the end of the tube exactly 12 inches above the center surface of the cultured cream.

(c) Insert the plummet, point downward, in the glass tube and hold in place with the smooth surface of the small board, maintaining a horizontal position against the bottom of the tube.

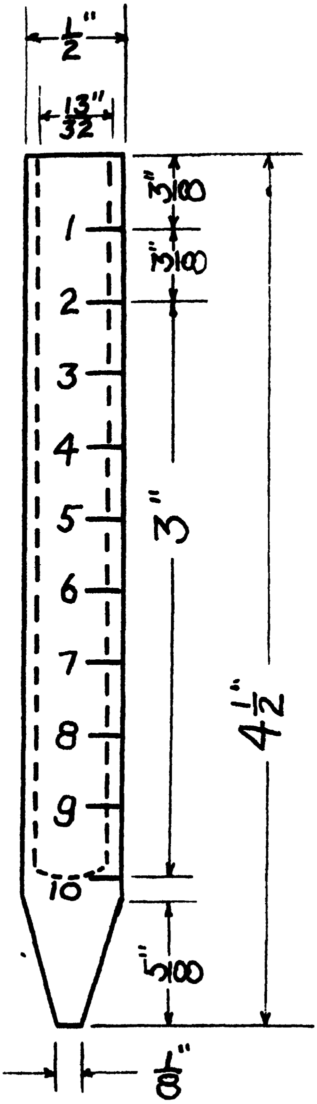

FIG. 1. Construction of plummet.

(d) Release the plummet by sliding the board quickly to one side.

(e) Lift the plummet from the cultured cream 5 seconds after its release.

(f) The cream will adhere to the plummet to a distance depending on the depth of penetration. Observe the cream line reading to the nearest quarter division of the scale. The cream line mark may not correspond to the true depth of penetration, due to the meniscus.

(g) An average of three tests is used as the measure of the body of the product. Use a different container of product for each test.

(h) The plummet should be washed and dried after each test.

TABLE 1

The body of cultured cream as measured by the plummet method

Plant no.	Batch no.	Plummet reading				Maximum deviation between individual sample containers
		Container no.			Av.	
		1	2	3		
1	1	7.50	7.25	7.25	7.33	0.25
	2	7.75	7.75	7.75	7.75	0.00
	3	7.25	7.50	7.50	7.42	0.25
	4	8.00	8.25	8.25	8.17	0.25
	5	7.75	8.25	8.00	8.00	0.50
	6	7.50	7.50	7.50	7.50	0.00
2	1	5.75	6.00	6.00	5.92	0.25
	2	7.00	7.25	7.00	7.08	0.25
	3	6.25	6.75	6.00	6.33	0.50
	4	7.00	7.25	6.50	6.91	0.75
	5	7.50	7.00	6.50	7.00	1.00
	6	7.25	6.75	7.00	7.00	0.50
3	1	5.00	5.50	5.25	5.25	0.50
	2	5.50	5.25	5.75	5.50	0.50
	3	6.75	7.25	6.50	6.83	0.75
	4	4.50	4.25	3.75	4.16	0.75
	5	4.75	4.25	4.50	4.50	0.50
	6	5.00	4.50	4.75	4.75	0.50
4	1	6.50	6.50	7.00	6.66	0.50
	2	8.00	8.50	8.00	8.16	0.50
	3	5.50	6.25	5.75	5.83	0.75
	4	7.75	8.00		7.87	0.25
	5	< 0	< 0	< 0		
	6	7.00	7.50	7.00	7.16	0.50
5	1	4.50	4.25	4.00	4.25	0.50
	2	6.00	5.75	6.50	6.08	0.75
	3	5.75	5.50	5.75	5.66	0.25
	4	5.00	4.50	4.50	4.66	0.50
	5	6.00	5.50	5.50	5.66	0.50
	6	6.00	6.00	5.50	5.83	0.50
6	1	3.25	3.50	3.25	3.41	0.25
	2	3.75	3.50	3.50	3.56	0.25
	3	4.00	4.25	3.75	4.00	0.50
	4	3.50	3.50	3.75	3.56	0.25
	5	3.50	3.50	3.50	3.50	0.00
	6	3.25	3.50	3.50	3.41	0.25

Data presented in table 1 give the body of cultured cream as produced by six dairy plants located in eastern United States. Examination of these data indicates that the variations existing between measurements of individual containers of the same lot of cultured cream are within the practical limits of controlled testing. A variation of ± 0.5 division is considered a suitable tolerance range. Daily tests on the body of cultured cream may be observed to indicate any variations from a predetermined standard.

The consensus of three experienced judges in correlating the visual viscosity of cultured cream with the plummet reading may be found in table 2.

TABLE 2

Relation of plummet readings to visual viscosity of cultured cream

Plummet reading	Visual viscosity
0 – 2	Very thin
2 – 4	Thin
4 – 6	Medium
6 – 7.5	Good
7.5– 8.5	Slightly heavy
8.5–10	Heavy
Over 10	Very heavy

SUMMARY

A simple method has been developed for measuring the body of cultured cream. The resistance of this product to the penetration of a plummet is taken as an indication of the body.

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THE FLAVOR, VOLATILE ACIDITY, AND SOLUBLE PROTEIN OF CHEDDAR AND OTHER CHEESE[1]

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The method of manufacturing Cheddar cheese has been known for many generations. About 50 years ago scientists began an intensive study of the factors affecting the flavor of this cheese with the idea that the source and identity of the flavoring substances could be established. Although much scientific knowledge has been accumulated from these studies, the specific flavoring materials are still unknown. The present trend toward the manufacture of Cheddar cheese from pasteurized milk has accentuated the need for this knowledge, as pasteurized milk cheese has less flavor and slightly different characteristics than that found in raw milk cheese.

The literature is voluminous and only a few references will be cited. The fundamental chemical changes that occur during the ripening process as known in 1891 were stated by Van Slyke (17). He wrote that there was a slow evolution of carbon dioxide from the casein or fat, or both. Volatile and nonvolatile fatty acids developed from the fat. The nitrogen compounds, especially casein, broke down into soluble compounds, some eventually becoming ammonia. Cured cheese was more alkaline than fresh cheese. The formation of the free fatty acids was the principal chemical change during ripening.

Van Slyke, Harding, and Hart (18) concluded from their study of rennet that this enzyme did not decompose protein into compounds that produced flavor in cheese. Suzuki, Hastings, and Hart (16) studied the origin and composition of steam distillate from cheese which contained the flavor compounds. They found bacteria to be the principal ripening agent. The lactose disappeared from cheese in 3 to 6 days but some of the lactates were fermented into volatile fatty acids, especially acetic and propionic. Butyric and caproic acids were derived from the fats and proteins. Succinic acid, alcohols, and esters also were present in the steam distillate.

Improved Cheddar cheese flavor has been reported with special cultures in Cheddar cheese making in addition to the usual lactic starter. Hucker and Marquardt (7) found that a *Streptococcus paracitrovorus* culture used with Hansen's commercial starter produced characteristic cheese flavor of superior quality from pasteurized milk. Proteolytic coccus cultures produced bitter flavor, as did the culture of *S. paracitrovorus* when used without starter. Hansen, Bendixen, and Theophilus (3) confirmed that cheese

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made with *S. paracitrovorus* alone became bitter, and they obtained similar results with *Streptococcus citrovorus.* However, the quality of cheese made from raw or pasteurized milk with lactic starters was not improved by the addition of *S. paracitrovorus* or *S. citrovorus.* Definite improvement in the quantity and quality of Cheddar cheese flavor was obtained by Lane and Hammer (8) when cultures of selected strains of *Lactobacillus casei* were used in pasteurized milk to which commercial lactic starter had been added. The protein decomposition was greater and the flavor improved due to this bacterium. More recently, Sherwood (13, 14) isolated 720 strains of lactic acid bacteria from pasteurized milk Cheddar cheese in New Zealand. The dominant organism was *Lactobacillus* (*Streptobacterium*) *plantarum,* with *Lactobacillus* (*Streptobacterium*) *casei* occurring less frequently. Betabacteria and betacocci were found in small numbers. Some strains of these bacteria produced good Cheddar cheese flavors. Studies in the laboratory and in factories verified Lane and Hammer's good results (8) with a special strain of *L. casei,* but best cheese flavor was obtained by the addition of 10 ml. of culture of *L. plantarum* to 80 gallons of milk to give an inoculation of about 12,000 organisms per ml. of pasteurized milk.

The rôle of the hydrolysis of milk fat and the development of volatile acids in relation to flavor has been emphasized by the studies of Lane and Hammer (9, 10, 11). The homogenization of milk for the manufacture of blue cheese improved the texture and increased volatile acidity and flavor. In Cheddar cheese the volatile acidity and flavor were greater for raw milk cheese than for pasteurized milk cheese. Homogenization of the milk or the addition of lipase from several sources generally produced rancid, bitter cheese, but these flavors tended to disappear with age. Desiccated mammary tissue added to cheese milk often produced desirable Cheddar flavor. Babel and Hammer (1) found that the lipase in mulberry juice or rennet extract often did improve the flavor of Cheddar cheese, but too much of the enzyme produced bitter flavor. Dahle and Watrous (2) based their new procedure for making Parmesan-type cheese on the fact that homogenization of the milk promoted the development of the rancid flavor and aroma desired in this grating cheese for flavoring spaghetti and soups.

Cheese ripening generally is followed by increased volatile fatty acids and increased soluble nitrogen. Both chemical changes are used to indicate the degree of ripening as associated with the development of flavor, and increased soluble nitrogen with the development of a mellow, waxy body. The usual steam distillation of cheese for volatile acids does not give correct results, according to Hiscox, Harrison, and Wolf (4, 5, 6) who developed a long, accurate method for estimating these acids. The accurate, rapid technic of Smiley, Kosikowsky, and Dahlberg (15) has made it possible easily to analyze commercial cheese for total volatile acidity and to compare such values with flavor scores and intensities.

This study was undertaken to show the relationship of total volatile acidity to flavor, and consideration also was given to several other chemical determinations, including soluble nitrogen.

METHODS

Five cheese manufacturers or processors in New York and Wisconsin were requested to select three Cheddar cheeses that represented mild, medium, and sharp flavors of excellent quality. Each sample was to be selected as being a typical good Cheddar cheese. The cheese selectors were cautioned to avoid choosing the cheese on the basis of age and to depend solely upon examination of the cheese. In one case the three cheeses received from one manufacturer were made at one plant.

As the 15 samples were received, they were classified as mild, medium, or sharp in flavor. After all had been received, they were arranged in order of the intensity of the Cheddar flavor by the authors working independently and then together. The samples were analyzed for volatile acidity, soluble protein, pH, moisture, and salt.

Samples of cheese of several varieties other than Cheddar were purchased at a retail grocery store. These samples were classified as to flavor intensity and analyzed chemically. They were rated on the basis of intensity of flavor within their respective varieties and also without regard to variety.

Finally, a manufacturer of Camembert cheese was asked to select several samples that were approximately the same age and had been treated similarly but which showed differences in the degree of ripeness and intensity of flavor. The four samples were scored and classified by the manufacturer and his comments were confirmed by the authors.

The total volatile acidity was determined by the method of Smiley, Kosikowsky, and Dahlberg (15), and the pH by the Beckman pH meter, using glass electrodes.

The solution of the soluble protein followed a technic based upon the method of Sharp (12). The principle of the method is to maintain the pH (approximately 5) and the salt content comparable to that of Cheddar cheese by a buffer salt solution while the soluble protein is being dissolved in the water. The soluble protein was extracted as follows: Three grams of cheese are weighed within 0.01 g. error and placed in a porcelain mortar. A small amount of extracting solution at 50° C. is added and the cheese is ground to a thick paste. Additional solution is added to dilute the paste. The dilute suspension of cheese is transferred to a 100-ml. flask. The mortar is rinsed with additional portions of the solution. The flask is placed in a water bath at 50° C., filled to the mark with extracting solution, and, with occasional shaking, maintained at this temperature for one hour. The solution is filtered through a fluted filter, and 50 ml. of the filtrate is placed in a Kjeldahl flask.

The soluble protein extraction solution is prepared as follows:

A. Stock solution

57.5	ml.	glacial acetic acid
136.1	g.	sodium acetate (3 H_2O)
47.0	g.	sodium chloride
8.9	g.	calcium chloride (anhydrous)

Add water to make 1 liter.

B. Extraction solution

Make 250 ml. stock solution up to 1 liter with water.

RESULTS

Selected Cheddar Cheese

The data on Cheddar cheese, presented in table 1, do not show any striking relationships of intensity of cheese flavor with any of the factors studied. In a general way it may be stated that the comments of the manufacturers of the cheese agreed well with those of the authors as to the intensity of the cheese flavor. The scores of the cheese showed that all samples were good Cheddar cheese but the scores were not related to intensity of flavor.

It was true that the two oldest samples of cheese (E1 and A2 which were 13 and 14 months of age) were the highest in flavor intensity. However, age of cheese cannot be segregated from temperature of storage, for curing cheese is a time-temperature problem and this temperature was not reported. This probably explains the reason that the cheese (B3 and B4) which rated third and fourth in strength of flavor were among the youngest, 2 and 3 months old, for the buyer of this cheese often force-cures it at about 60° F., as compared with the usual storage temperature of 32 to 35° F. For this reason and others, flavor intensity and age of cheese were not associated closely enough to be able to state that a given cheese was strong in flavor because it was old (10 to 12 months) or that another was mild in flavor because it was young (2 to 4 months).

The principal object of this study was to establish the relationship between the intensity of Cheddar cheese flavor and total volatile acidity. There were two samples with volatile acidities below 20 ml. N/10 acid per 100 g. of cheese. These samples were 13 and 7 months old and were first and fourteenth in flavor intensity. There were three samples with volatile acidities over 40. These samples were 14, 2, and 2 months old and were second, seventh, and eleventh in intensity of flavor. With the two cheeses of lowest volatile acidity representing the most and next to the least flavor intensity, and the cheeses of highest volatile acidity also scattered as to flavor intensity, it is evident that a sample of Cheddar cheese could not be classed as to flavor intensity on the basis of total volatile acidity. There appears

TABLE 1

The relationship of flavor intensity, volatile acidity, soluble protein, and other constants in commercial Cheddar cheese of good flavor

Sample no.*	Age of cheese	Raw or past. milk	Comment on flavor		Authors' score	Order of flavor intensity	Volatile acidity†	Soluble protein	pH	Mois- ture	Salt
			Mfgr's.	Authors'							
	(*mos.*)							(%)		(%)	(%)
E1	13	Raw	Sharp	Sharp	94	1	16.8	8.40	5.10	35.56	1.33
A2	14	Past.	Sharp	Sharp	92	2	48.0	8.88	5.20	35.91	1.41
B3	3	Raw	Sharp	Sharp –	92	3	29.2	5.56	5.00	39.01	1.87
B4	2	Raw	Medium	Sharp –	91	4	32.6	5.53	5.06	37.73	1.45
C5	12	Raw	Medium	Medium +	90.5	5	18.8	8.17	5.33	33.60	2.00
D6	12	Past.	Sharp	Mild +	93	6	24.0	7.27	5.51	35.95	2.13
B7	2	Raw	Medium	Medium +	90	7	40.5	4.92	5.18	37.49	1.39
C8	9	Raw	Sharp	Medium	91.5	8	25.8	7.47	5.10	35.68	1.98
B9	3	Raw	Mild	Medium	91	9	23.2	7.12	5.30	39.99	1.58
D10	9	Past.	Medium	Medium –	92	10	24.0	5.81	5.28	37.25	1.48
A11	2	Raw	Mild	Mild +	92	11	40.8	4.82	5.00	34.26	1.23
A12	10	Past.	Medium	Mild +	91.5	12	28.3	7.15	5.08	33.97	1.03
D13	4	Past.	Mild	Mild +	92	13	25.0	4.53	5.39	37.79	2.05
E14	7	Past.	Mild	Mild	92	14	13.7	6.68	5.25	37.53	1.48
C15	3	Raw	Mild	Mild	92	15	21.7	5.56	5.49	35.64	1.48

* The letter refers to the cheese manufacturer or processor who selected the samples.

† Ml. N/10 acid per 100 g. cheese.

to be almost no general trend in the relationship between volatile acidity and flavor intensity.

The data on the relationship between soluble protein and flavor intensity did not happen to be so strikingly negative as was the case for total volatile acidity. Samples E1 and A2, with the greatest amount of soluble protein, 8.40 and 8.88 per cent, respectively, were oldest and ranked first and second in intensity of Cheddar cheese flavor. However, the relationship between the two factors appeared to end with these two samples. Samples with less than 5 per cent soluble protein were seventh, eleventh, and thirteenth in flavor. Samples with 5 to 6 per cent soluble protein were third, fourth, tenth, and fifteenth in flavor intensity. There was no obvious trend in the relationship between these two factors.

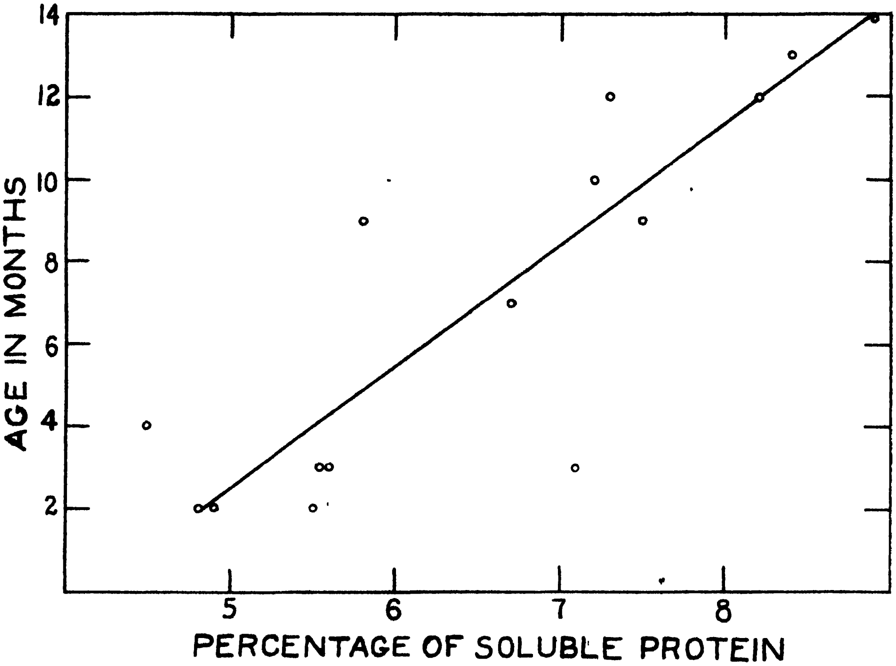

FIG. 1. Relation of percentage of soluble protein to age of Cheddar cheese.

The percentage of soluble protein increased with increased age of the cheese (fig. 1). This trend had some exceptions but it was reasonably good considering the varied character of the cheese. A similar relationship did not exist between total volatile acidity and age of the cheese.

There is nothing in the data to suggest any correlation of flavor intensity with the pH, moisture, and salt content of the cheese.

Miscellaneous Cheese Varieties

The miscellaneous varieties of cheese purchased at a grocery store gave

TABLE 2

The relationship of volatile acidity, pH, and the quality and intensity of flavor of various types of cheese

Cheese variety	Cheese characteristics			Volatile acidity*	pH
	Relative intensity of flavor of all cheese	Flavor in each individual cheese class			
		Intensity of flavor	Quality remarks		
Camembert	1	Strong	Overripe	39.9	7.43
Blue	2	Medium	Good	40.4	6.00
Kaukauna Klub Cheese Food	3	Medium	Good	30.8	5.36
Liederkranz	4	Strong	Good	102.8	7.27
D'Oka (Trappist)	5	Strong	Fair	49.2	5.90
Chantelle	6	Medium	Fair	31.4	5.23
Swiss	7	Mild	Good	49.5	5.57

* Ml. N/10 acid per 100 g. cheese.

very interesting data (table 2). A high-flavored, overripe, soft Camembert cheese with an ammonia odor was rated as having the strongest flavor. It was alkaline in pH. A green, mild but good-flavored Swiss cheese was rated lowest in flavor; yet the total volatile acidity of the Swiss was greater than that of the Camembert. A blue cheese of medium-strength flavor of good quality was rated second in flavor intensity and its volatile acidity was the same as that of the Camembert. A strong-flavored Liederkranz cheese that was slightly alkaline contained more than twice as much volatile acidity as any other cheese; yet it was not the strongest-flavored cheese. Its flavor was very much the same as the D'Oka cheese with only half the volatile acidity.

It is appreciated that this attempt to rate cheese of different varieties on intensity of flavor is subject to criticism. The flavors are so different that intensities cannot be compared closely. After making allowances for such discrepancies, there can be no doubt concerning the lack of relationship between total volatile acidity and intensity of flavor.

TABLE 3

Analysis of four domestic Camembert cheeses from different lots made in the same plant

Cheese	Age	Manufacturer's grade		Volatile acidity*	Soluble protein	pH	Moisture
		Score	Comments				
	(days)				(%)		(%)
1	48	20.0	Mild flavor Slight flat Poor breakdown	20.4	5.84	6.63	52.03
2	45	21.0	Mild flavor Fair breakdown	16.1	6.00	6.78	53.56
3	48	21.5	Fair flavor Fair breakdown	18.6	6.53	7.01	51.00
4	47	22.0	Good cheese	21.4	6.41	6.95	51.93

* Ml. N/10 acid per 100 g. cheese.

Selected Camembert Cheese

The ripening of Camembert cheese is sometimes subject to unexpected flavor variations which might be associated with total volatile acidity and soluble protein. It is recognized that the softening of the cheese is dependent upon protein solubility. A manufacturer of Camembert selected four cheeses of approximately the same age but with marked variation in the amount of good Camembert flavor (table 3). The manufacturer's score and comments on the cheese were used for comparisons with analysis.

The total volatile acidities of the cheese with the least flavor and with the most flavor were practically identical. The percentage of soluble protein and the pH value increased as the flavor increased in intensity and the cheese became softer in body. It is possbile that the relationship between intensity of flavor and changes in soluble nitrogen and pH were incidental to the obvious change in the body of the cheese, which must be related to these changes.

DISCUSSION

The tendency toward the manufacture of cheese from pasteurized milk has emphasized the lack of knowledge of those chemical entities which are responsible for the characteristic flavor of cheese. The literature of the last century gave the two major chemical changes that occur during ripening, namely, the increase in total volatile and nonvolatile acidity, and the increase in soluble protein. The emphasis was given to volatile acidity more than to the decomposition products of protein. Many investigators have endeavored to increase cheese flavor by the bacteria and enzymes which affect the fat and the casein.

This research has established that the magnitude of these chemical changes is not related to the intensity of the flavor of Cheddar cheese. The increase in total volatile acidity and soluble nitrogen during ripening is interesting and valuable information to obtain on cheese ripening, but flavor development is not related to these characteristics. This means that much of the research on the development of cheese flavor has been based upon a fundamental misconception. It may be that the cheese flavor is a result of the decomposition of milk fat or protein, but, if such is the case, the flavor compounds are developed without regard to the total changes in these two materials. In other words, if the flavor compounds of Cheddar cheese are volatile fatty acids, then the quantity of the flavor-producing acids is not related to total volatile acidity. It has been assumed fallaciously that these two were synonymous, as the volatile acids contain most of the cheese flavor.

Cheese flavoring compounds are developed during ripening incidental to the common chemical changes with which investigators often are concerned. This means there is need for a widening of the concept behind the studies of cheese flavor. More rapid progress may be made by new manufacturing technics or by identification of the flavor compounds. There is real hope for producing higher flavors in pasteurized milk cheese. For example, sample A2 was a pasteurized milk Cheddar cheese that possessed second highest flavor and the highest volatile acidity and soluble protein. This cheese may have been an exception for the plant that made it, but the fact is that it was produced.

As cheese ripens there is an increase in flavor, volatile acidity, and soluble protein. The lack of any correlation among these progressive changes in Cheddar cheese, except for a general relationship between age and soluble protein, does not disprove their occurrence. Instead, it emphasizes that there are a number of factors which affect each of these changes in varying degrees and partially obscure the effect of age alone. The direct relationship between age and soluble protein might be explained as being due chiefly to the action of rennet as affected by time, with other factors being less important in their total effects. The effect of microorganisms on the development of flavor is of major importance and is influenced greatly by a number of factors other than variations in time.

CONCLUSIONS

Cheese manufacturers and processors in New York and Wisconsin selected 15 Cheddar cheeses with typical good flavors that were mild, medium, and sharp in intensity. The cheese varied from 2 to 14 months of age. There were no relationships among intensity of flavor, total volatile acidity, soluble protein content, pH, and age of the cheese, except for a direct relationship between age and soluble protein. The flavor compounds were distilled with the volatile acids but apparently were not directly correlated as to total amounts

No relationship was found between intensity of flavor and total volatile acidity in seven different varieties of cheese secured at a retail store.

Four samples of Camembert cheese made in one factory were selected to show difference in flavor and texture. There was no relationship between intensity of flavor and total volatile acidity but there was a direct relationship between flavor intensity of this cheese and its soluble protein and pH values.

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THE VALUE OF SUPPLEMENTARY VITAMIN FEEDING IN THE REARING OF DAIRY CALVES

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Widespread interest in the vitamin needs of dairy calves was aroused through several reports (5, 6, 9, 10) that supplementary vitamin feeding during the first few weeks of life prevented so-called "nutritional scours", lessened navel infection, and reduced the death rate. The studies pointed to vitamins A and C and nicotinic acid as essential components of the ration of the young calf. The reported beneficial effects of vitamin supplementation were so striking that it was considered desirable to test the value of such a procedure in a dairy herd in which good feeding practices are followed throughout the year. On the basis of a number of published studies showing a relation between the character of the ration and the vitamin content of colostrum milk, it was assumed that the mother's ration during the gestation period also may affect the store of vitamins in the body of the calf at birth. Recent reports (3, 11, 14) tend to substantiate the validity of such an assumption.

A further assumption based on numerous calf-raising trials is that the most critical period in the life of the hand-fed dairy calf is within the first 30 days after birth. An early investigation in the feeding of dairy calves conducted at this Station (1) indicated that the character of the ration during the first 3 weeks is of prime importance. The data reported in this paper, therefore, cover only records up to 30 days of age. Histories of 299 calves, approximately one half of them males, are summarized.

PROCEDURE

The study was carried out with calves born in the Station dairy herd. The cows in this herd were fed hay, silage, and grain mixture throughout the year, with limited access to pasture during the growing season. The quality of all feeds, as a rule, was much above average. Alfalfa hay was the chief dry roughage, with smaller amounts of red clover hay and Korean lespedeza hay. The calves usually were left with their mothers for 2 days after birth, but some weak calves were left as long as 4 days. After separation from their dams they were kept in special quarters at two different locations. Feeding of whole milk (Holstein) was continued throughout the 30-day period of observation covered by these records. The calves were given free access to adequate amounts of red clover hay and a grain mixture. Liveweights were taken as early as possible after birth. However, some calves were born when no attendant was present, and probably a part of these had opportunity to nurse before they were weighed. These weights, therefore,

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are termed first-day weights, rather than birth weights. Liveweights also were taken at 15 days and at 30 days of age.

The vitamin supplements were supplied in two forms. Nopco special A and D feeding oil with a potency of 2,000 units of A and 800 units of D per gram was supplied at the rate of approximately 2 ml. per head daily to all calves at one barn for the first half of the experimental period and none was given during the second half of the period. At the other barn the practice was reversed. Approximately equal numbers of calves were included in the groups fed oil and no oil. The other form of supplement consisted of vitamin capsules, the formulae for which were as follows:

	Black capsule	*White capsule*
Vitamin A, U.S.P. units	5,000	5,000
Vitamin D (Irradiated ergosterol), U.S.P. units	500	500
Nicotinic acid, mg.	50	50
Vitamin C (Ascorbic acid), mg.	250	

Random selection of the calves to be fed the vitamin capsules was achieved by following the rule that the first calf born was to be fed vitamin capsules, the next one to be given no vitamin capsules, and so on. Thus one half of the calves were fed vitamin capsules and the remainder served as controls. One capsule containing vitamins A and D, nicotinic acid, and ascorbic acid was given daily per calf from 1 to 10 days of age and one capsule containing only vitamins A, D, and nicotinic acid was administered daily from 11 to 30 days of age.

Four experimental groups were established. Group I received no vitamin supplement, group II received the vitamin capsules, group III received the vitamin A and D oil supplement and group IV received both the vitamin capsules and the oil supplement.

An individual record sheet for each calf was posted on a bulletin board and records kept of the vitamins and milk fed, liveweights, condition of the calf, and, in the event that scours occurred, the treatment given and the duration of the ailment. The trials reported in this paper began early in 1944 and extended through September, 1946.

RESULTS

A summary of the records of all calves which survived the 30-day observation period is given in table 1. Characteristic breed differences were noted in the first-day weights of the calves. The average weights were: Ayrshires, 76 lbs.; Brown Swiss, 98 lbs.; Guernseys, 66 lbs.; Holsteins, 96 lbs.; and Jerseys, 50 lbs. The average gains in liveweight were somewhat greater for the Brown Swiss and Holsteins than for the other breeds. All of the average gains, however, were satisfactory for the breeds represented with the exception of those for the Guernseys in group II. This group included three calves which made very small gains. The average 30-day

TABLE 1

Effects of vitamin supplementation on liveweight gains and incidence of scours in dairy calves

Breed*	No. of calves	Av. values per head			Milk fed	Scours		
		First-day weight	Gain in weight			Number of cases	Av. age began	Av. duration
			To 15 days	To 30 days				
		(*lbs.*)	(*lbs.*)	(*lbs.*)	(*lbs.*)		(*days*)	(*days*)
I. Calves fed no vitamin supplement								
A	9	79.1	7.0	18.1	258	3	9	2
B.S.	5	104.9	9.4	20.3	233			
G	7	62.5	7.9	19.9	202	1	12	2
H	35	95.6	9.3	21.0	234	8	7	4
J	12	45.3	8.5	17.7	173	4	9	2
All	68	81.8	8.7 ± 0.32	19.9 ± 0.41	223	16	8	3
Coefficient of variability .. .			43.9 ± 0.36	24.8 ± 0.21				
II. Calves fed vitamin capsules								
A	12	75.4	6.9	18.7	238	6	12	5
B.S.	10	97.8	10.1	21.0	214	2	6	3
G	14	62.6	4.5	12.9	183	3	9	3
H	32	97.7	8.6	20.8	234	7	8	3
J	8	49.3	8.2	18.1	184	1	9	3
All	76	82.6	7.7 ± 0.42	18.8 ± 0.97	217	19	9	4
Coefficient of variability ..			70.0 ± 0.54	45.0 ± 0.35				
III. Calves fed vitamin A and D oil concentrate								
A	7	75.7	9.9	23.6	244	1	19	3
B.S.	8	94.2	12.9	26.4	221	2	8	2
G	6	70.3	8.5	18.3	218	1	14	3
H	36	98.1	10.8	25.1	260	10	10	2
J	9	51.8	9.6	20.9	187	6	6	2
All	66	86.4	10.6 ± 0.35	23.9 ± 0.55	239	20	9	2
Coefficient of variability			39.6 ± 0.33	27.3 ± 0.23				
IV. Calves fed vitamin capsules plus vitamin A and D oil concentrate								
A	4	69.8	10.7	23.3	242	1	21	2
B.S.	8	97.4	11.1	25.6	245			
G	7	71.9	7.8	21.6	216	1	19	1
H	36	91.7	10.1	26.2	261	7	6	3
J	9	53.3	9.3	19.7	198	2	4	2
All	64	83.5	9.8 ± 0.36	24.5 ± 0.57	243	11	8	2
Coefficient of variability			42.8 ± 0.37	27.2 ± 0.24				

* A, Ayrshire; B.S., Brown Swiss; G, Guernsey; H, Holstein; J, Jersey.

gain for the 11 other Guernseys in this group was 16 lbs., a satisfactory figure. The gains for the calves in groups III and IV were larger than those for groups I and II, but the amounts of milk fed to groups III and IV also were larger.

The four groups of calves were similar with respect to the number of cases of scours, age at which the attacks of scours occurred, and the duration of the ailment.

A summary of the records of calves which survived the 2-day nursing period and were started on hand feeding but which failed to complete the 30-day experimental period is given in table 2. Records of calves born dead and of those that died within 48 hours are not included. Death was attributed to a number of causes, among which pneumonia alone and also scours accompanied by or followed by pneumonia were important. There was little difference between the groups, however, in the number of calves affected. Only two of the calves in group I died at less than 12 days of age. One calf in group I and one calf in group IV died of pneumonia on the 30th day. It was noted that in a considerable number of cases scours did not occur until the calves had reached an age of 2 to 3 weeks.

TABLE 2

History and apparent cause of death of calves which failed to survive the 30-day observation period

	Group and kind of vitamin supplementation			
	I None	II Vitamin capsules	III A and D feeding oil	IV Capsules and A and D feeding oil
No. of calves	8	6	6	5
Average age at death, days	16	9	14	12
Death attributed to:				
Accidents		1		
Indigestion and scours	1	1*	1	1
Pneumonia	1		2	2
Scours and pneumonia	3	1		
Too weak to stand or nurse	1	1	1	2
Very weak at birth	2	2	1†	
Weak at birth; died from bloat			1	

* Mother had mastitis.
† Mother had preparturient mastitis.

DISCUSSION

Considerable variability in liveweight gains occurred among the calves of the four groups. The gains to 15 days of age showed greater variability than the gains to 30 days. This was an expected difference because more cases of scours occurred prior to 15 days of age than between 15 and 30 days and, also, some calves which were weak at birth and made a slow start made good gains after 15 days. The variability of the gains to 30 days of age was, surprisingly, somewhat less than that found at this Station for pasture-fed yearling dairy heifers (7).

The use of vitamin supplements was not effective in reducing the number of cases of scours, the duration of the ailment, and the mortality rate below those of the controls (group I). It appeared that weakness at birth and inability to stand or nurse, the presence of infectious mastitis in the udder of the mother, accidents, and exposure to cold followed by pneumonia were important contributing causes of illness and death.

The use of capsules containing vitamins A and D, together with nicotinic acid and ascorbic acid, was no more effectual in promoting gains in weight or in reducing scours than the use of the special feeding oil containing only vitamins A and D, as judged by the records of calves included in groups II, III, and IV. It is concluded, therefore, that the supplementation of the whole milk ration with ascorbic acid and nicotinic acid was ineffectual in promoting the well-being of the calves in this herd. This finding is in agreement with recent investigations at this Station (4, 12) which showed that ascorbic acid and nicotinic acid are not required by the dairy calf. On the other hand, the same technics used in these investigations disclosed the fact that riboflavin is required by dairy calves (13). These several experiments lend no support to the inference given in a recent report (2) that the supplementary effect of nicotinic acid is dependent upon the level of vitamin A intake.

Our investigations lead us to the conviction that a measure of the progress of the calf during the first 30 days after birth is a better index of nutritional status in early life than is any other period. The character of the ration after 30 days of age may be much more varied than prior to that time. The consumption of high-quality hay and special grain mixture, both of which may be good sources of vitamins and other nutrients, tends to correct nutritional deficiencies incurred during the first few weeks of life. In a recent report of controlled experiments with young calves (8), it is shown that 35 of 57 calves developed cases of scours, but only one of the cases that occurred after 4 weeks of age affected a calf not previously afflicted with the ailment.

Our results do not preclude the possibility that nutritional deficiencies may have been responsible for the onset of scours and other ailments in the case of some calves, particularly those which were too weak at birth to nurse and those which were not strong enough to obtain adequate amounts of colostrum milk. Further, the desirability of vitamin A and D supplementation in the rations of calves born in herds where the rations are low in vitamin value is not disproved. It is assumed on the basis of recent reports (3, 11, 14) that one of the important reasons that vitamin supplementation was of questionable value in our trials was that the cows in the herd were at all times well fed and that such feeding provided vitamin reserves in the new-born calf.

SUMMARY

Individual records were kept of 299 calves of the Station dairy herd. These were divided at random into four groups to determine the value of vitamin supplementation of the ration during the first 30 days, the critical period in the life of the calf.

The vitamin supplements were of two kinds. One consisted of vitamin capsules containing vitamins A and D, together with ascorbic acid and nicotinic acid. Ascorbic acid was omitted after 10 days of age. The other was a special feeding oil containing vitamins A and D.

The criteria used in evaluating the results were gains in liveweight to 15 days and to 30 days of age, number of cases of scours, age at which scours occurred, duration of scours, number of deaths, and cause of death in those which failed to survive the 30-day period of observation.

The use of vitamin capsules containing ascorbic acid and nicotinic acid in addition to vitamins A and D was not found superior to supplementation with only vitamins A and D, and vitamin supplementation on the whole was of doubtful value, as shown by the bases of measurement used.

ACKNOWLEDGMENT

The formulae for the vitamin capsules used in these trials were worked out by the Gelatin Products Corporation, Detroit, Michigan, in consultation with the senior author. The capsules were furnished through the courtesy of the above-named corporation. The assistance of members of the staff of the College of Veterinary Medicine of the University of Illinois in making diagnoses and post-mortem examinations is gratefully acknowledged.

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THE EFFECT OF CONTINUOUS INTRAVENOUS FEEDING OF VARIOUS SUBSTANCES UPON THE SECRETION OF MILK FAT[1, 2]

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Kaufmann and Shaw (7) showed that carbohydrate when fed as the sole diet provides the precursors of the lower fatty acids of milk fat. As marked hypoglycemia does not in itself produce a decrease in the lower fatty acids (16), it was suggested that dietary carbohydrate acts indirectly either by exerting a sparing action on the utilization of the precursor substances by other body tissues or by being converted into the necessary precursors in the rumen by the action of microorganisms.

It appeared that the best test of the alternative hypotheses would be that of the intravenous and abomasal feeding of glucose to fasted cows and goats. Accordingly, methods were devised for the continuous intravenous and abomasal feeding of ruminants. Using these methods, a study was made of the effect of the administration of glucose and other substances upon the secretion of the lower fatty acids of milk fat.

METHODS

The approach to the problem of ascertaining the blood precursors of the lower fatty acids was similar to that employed in earlier studies (7) in which advantage was taken of the well-known fact that inanition decreases the lower fatty acid content of milk fat. As a marked decrease in the lower acids always occurs within a period of 24 hours of fasting, it was assumed that if the proper precursor or precursors were fed either intravenously or abomasally to a fasted lactating ruminant, the usual decrease in these lower acids would be retarded or prevented. Several substances were administered in this fashion and the effect upon the lower fatty acids of milk fat noted, as shown by the Reichert-Meissl value and, in some cases, the Polenske value. Iodine numbers also were obtained on the milk fat.

In order to maintain a relatively high level of the various substances in the blood and thus insure that these substances would be available in above-normal quantities for the possible synthesis of milk fat, it was deemed

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advisable to administer such substances continuously for a period of 24 or more hours. The technic used for intravenous alimentation proved so satisfactory that it is described in some detail.

The technic consists of fixing a no. 8 or no. 10 rubber catheter in the subcutaneous abdominal mammary vein and connecting this catheter to a drip bottle by means of a gum rubber tube. It appeared advisable to anesthetize the cow. Accordingly, from 3 to 5 g. of nembutal were injected into the jugular vein at a rapid rate. The animal may be thrown or allowed to fall in a well-bedded stall. It is sometimes necessary to inject an additional gram or so after the cow is in a prone position, particularly if the cow is allowed to become excited. In most cases the operation can be completed without necessitating more than one additional injection.

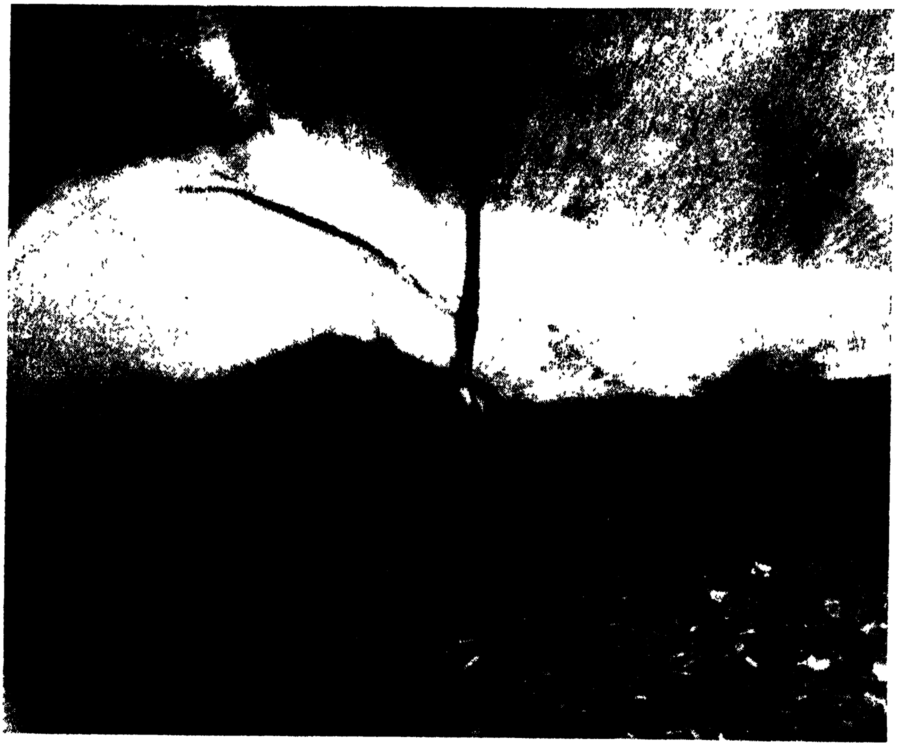

FIG. 1. The rubber catheter fixed in the vein for continuous intravenous feeding.

A small area of the skin, which has been clipped previously, is shaved and an incision approximately 3 cm. in length is made in the skin over one of the subcutaneous abdominal mammary veins. The muscles covering the vein are dissected carefully until a small area of the vein is exposed and a portion is picked up by means of a hemostat. A transverse incision is made in the vein close to the hemostat by means of a small scissors, and approximately two-thirds of the length of the catheter is inserted into the vein. If the opening in the vein is sufficiently small so that the catheter fits snugly, hematoma will be held to a minimum. Prior to beginning the operation, the clamp on the tube leading from the drip bottle is loosened so that fluid is dripping slowly from the end of the catheter when it is inserted. Three to four sutures are sufficient to close the incision. The first suture is made close to the catheter, knotted and then tied around the catheter to hold it in place. The catheter is shown in place in figure 1.

The tube leading to the drip bottle is attached to the skin by means of sutures, one in the region of the flank and the other over the last rib, approximately half the distance to the backbone. After the cow regains her feet, a wide cloth surcingle is tied around the midsection in order to cover the catheter and the tube leading to the drip bottle. The rubber tube is tied firmly to the surcingle in the region of the loin so that all pull on the tube will be on the surcingle and not on the catheter. From the surcingle the tube passes through a fixed pulley 18 to 24 inches above the backbone of the animal. A sliding weight is placed on the tube between the pulley and the drip bottle with sufficient slack so that the animal can move about and

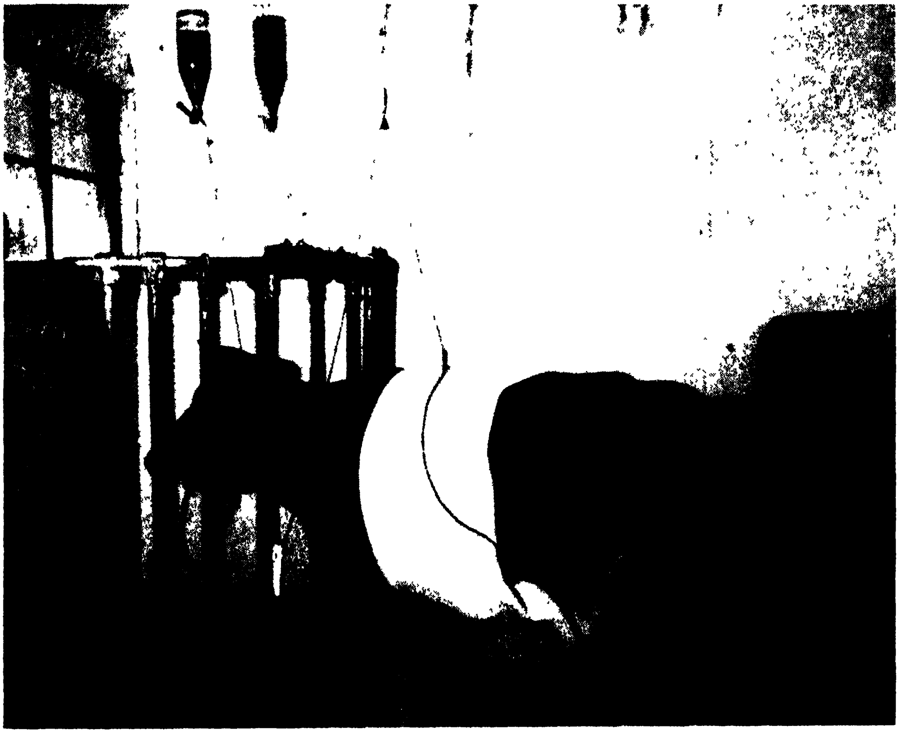

FIG. 2. The arrangement by which continuous intravenous feeding was effected without discomfort to the animal.

lie down and get up at will. The details of this arrangement are shown in figure 2.

In the initial studies, goats were fed abomasally by means of a Pezzar catheter fixed in the abomasum and attached to a drip bottle in the manner described above. The abdominal cavity was opened along the ventral midline just posterior to the sternum and the catheter fixed in the abomasum by means of a purse-string suture. The catheter was brought to the exterior through an opening made between two ribs approximately halfway between the midventral line and the dorsal midline, after which the incision along the ventral midline was closed.

The iodine numbers (Hanus) and the Reichert-Meissl and Polenske values were determined on milk fat according to the methods outlined in the Official and Tentative Methods of Analysis (1).

RESULTS

Two goats in milk were fed abomasally. One received glucose and the other triacetin. Nine cows were fed by the intravenous route. The substances administered included glucose, "Pepticase"[3] (a protein hydrolysate), sodium oleate, sodium acetate, and sodium butyrate. The animals usually received grain but no roughage in the last feeding prior to the beginning of the period of intravenous or abomasal feeding.

Fat constants were determined on the purified fat prepared from milk obtained at the beginning and at the usual milking intervals during the experimental periods.

Glucose. Glucose was fed abomasally to a fasted goat and intravenously to two fasted cows, one of which received a protein hydrolysate in addition to the glucose. That the blood glucose level was maintained above normal is evidenced by the fact that in each of the three cases the glucose intake was maintained just slightly below that which produced a mild glucose shock. It is apparent, therefore, that no blood glucose deficiency existed during the fasting periods. In experiment 2 a total of 1985 g. of glucose was injected in 31 hours. It will be noted from the data on experiments 1, 2, and 3 in table 1 that neither the decrease in the Reichert-Meissl values nor the increase in the iodine values was prevented by the administration of glucose.

Protein hydrolysate. Pepticase was administered to one cow by the intravenous route to test the possibility of certain amino acids being involved in the synthesis of the lower fatty acids of milk fat. As blood glucose does not appear to be one of the precursor substances, glucose was administered with the protein hydrolysate with the object of providing additional energy so that the protein hydrolysate would not be utilized too rapidly for energy purposes. During an injection period of 31 hours, a total of 464 g. of Pepticase and 1392 g. of glucose was administered. The Reichert-Meissl value

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[3] Supplied through the courtesy of Sheffield Farms Company.

TABLE 1

The effect of intravenous and/or abomasal feeding of glucose, sodium oleate, and a protein hydrolysate to fasted ruminants upon the character of the fatty acids of milk fat

Animal no.	Method of adminis-tration	Test substance	Hours after regular feeding	Grams of test sub-stance fed (cumulative)	Milk fat constants			Comments
					R.M.V.*	P.V.†	I.N.‡	
1 (goat)	Abomasal	Glucose 15%			22.94		34.74	Normal milk
			18					Started expt. feeding
			24	325	20.06		39.12	
			36	496				
			59	775	17.92		43.12	
2 (cow)	Intraven.	Glucose 20%			24.44		21.95	Normal milk
			23					Started infusion
			39	855	21.49		26.82	
			51	1340	17.07		27.62	
			63	1741	16.73		29.00	
			70	1985	18.29		27.61	
3 (cow)	Intraven.	Glucose (g) 15% and "Pepticase" (p) 5%			24.83		22.08	Normal milk
			22					Started infusion
			39	558 (g) 186 (p)	20.32		28.09	
			49	870 (g) 290 (p)	16.68		27.34	
			63	1188 (g) 396 (p)	15.10		24.46	
			70	1392 (g) 464 (p)	14.99		27.37	
7 (cow)	Intraven.	Sodium oleate 5.5%			25.19	2.21	37.93	Normal milk
			17					Started infusion
			33	150	21.65	1.35	44.05	

* R.M.V. = Reichert-Meissl values.

† P.V. = Polenske values.

‡ I.N. = Iodine numbers (Hanus).

decreased gradually from 24.83 to 14.99. The iodine value increased from 22.08 to 27.37. These values are fairly representative of changes which would be expected from inanition alone.

Oleic acid. After fasting a cow for 17 hours, 150 g. of sodium oleate in a 5.5 per cent solution were injected by the intravenous route within the following 16 hours. The experiment was terminated when excessive hemoglobinuria was observed. The data presented in table 1, experiment 7, do not indicate that oleic acid had any influence in arresting the changes in the milk fat constants which are typical of fasting.

Acetic acid. Four experiments were conducted to study the possible rôle of acetic acid in the synthesis of the lower fatty acids of milk fat. In one experiment acetic acid was administered abomasally to a milking goat as triacetin. Three cows received sodium acetate intravenously, one receiving sodium butyrate with the acetate. It is evident from the experiments in which acetate was administered alone (experiments 4, 5, and 6, table 2) that the intravenous injection of acetate did not prevent the changes in the milk fat which are typical of fasting.

The amounts of acetate administered are believed to be considerably in excess of that which is normally absorbed into the circulatory system after being produced in the rumen. In experiment 6, a total of 1984 g. of acetate was injected within 22 hours. This experiment was terminated when the cow suffered a temporary collapse resembling tetany. The collapse apparently was due to an alkalosis caused by the metabolizing of the acetic acid, thus freeing an excess of sodium ions in the blood.

Butyric acid. Four cows received sodium butyrate intravenously. Two of the cows received butyrate alone during a period of complete fasting, one received a combination of butyrate and acetate while being fasted, and one received butyrate while on full feed. These animals (nos. 9, 10, 11, and 12, table 3) had been receiving a ration of mixed hay, corn silage, and a 16 per cent concentrate prior to being placed on experiment. To avoid any possible effects of inanition, all four were fed in excess of requirements for a week prior to the experiment. One of the four, no. 12, was fasted without receiving injections of any kind in order to serve as a control for the other three. The fifth cow, no. 8, had been on a regime completely different from the others.

In the first test of butyrate, the results were startling. Cow no. 8 received a total of 1057 g. of sodium butyrate during a period of 29 hours. Not only was the Reichert-Meissl value of the milk fat maintained, but the Polenske value, representing primarily the octanoic and decanoic acids, actually increased from a normal of 2.40 and 2.36 to 2.89 and 2.75. These fat samples were checked repeatedly, without any substantial change in the original values. It will be noted, however, that the iodine values increased by about the same magnitude as that of the milk fat of the control cow.

TABLE 2

The effect of intravenous and/or abomasal feeding of triacetin and sodium acetate to fasted ruminants upon the character of the fatty acids of milk fat

Animal no.	Method of administration	Test substance	Hours after last regular feeding	Grams of test substance fed (cumulative)	Milk fat constants			Comments
					R.M.V.*	P.V.†	I.N.‡	
4 (goat)	Abomasal	Triacetin 5%			22.52		28.86	Normal milk
			17					Started expt. feeding
			24	18	18.75		36.01	
			35	35	16.07		36.22	
5 (cow)	Intraven.	Sodium acetate 15%			19.02		23.92	Normal milk
			17					Started infusion
			24	165	15.64		33.33	
			37	333	13.32		33.69	
			49	668	11.99		38.73	
6 (cow)	Intraven.	Sodium acetate 20%			23.94	1.24	43.25	Normal milk
			19					Started infusion
			41	1984	18.57	0.88	46.14	Symptoms of alkalosis

* R.M.V. = Reichert-Meissl values.

† P.V. = Polenske values.

‡ I.N. = Iodine numbers (Hanus).

TABLE 3

*The effect of intravenous feeding of sodium butyrate and sodium butyrate plus sodium acetate to cows upon the character of the fatty acids of milk fat**

Cow no.	Test substance	Hours after last regular feeding	Grams of test substance fed (cumulative)	Milk fat constants			Comments
				R.M.V.†	P.V.‡	I.N.**	
8	Sodium butyrate 12.6%			25.17	2.40	33.03	Normal milk
		7					Started infusion
		11½	100	25.31	2.36	32.14	
		26	589	26.44	2.89	34.84	
		36	1057	25.05	2.75	38.21	
9	Sodium butyrate 12.6%			31.10	3.35	33.90	Normal milk
		9					Started infusion
		38	1184	25.02	2.20	42.85	
		49½	1396	20.83	2.17	43.00	Symptoms of alkalosis
10	Sodium acetate 14% (a) plus sodium butyrate 12.6% (b)			31.44	2.81	33.55	Normal milk
		8					Started infusion
		15	361 (a) 322 (b)	30.15	3.11	35.07	
		30	1092 (a) 975 (b)	24.02	2.14	39.80	
11	Sodium butyrate 12.6% plus normal ration			28.98	3.61	27.85	Normal milk
		4					Started infusion
		11	136	28.92	3.40	28.35	
		38	786	29.45	3.70	26.20	
12	None			26.89	2.82	33.35	Normal milk
		13		26.35	3.34	36.90	Complete fasting
		37		17.61	0.92	42.65	

* Cows 9, 10, 11, and 12 received identical rations considerably in excess of requirements for one week prior to the experimental periods.

† Reichert-Meissl values.

‡ Polenske values.

** Iodine numbers (Hanus).

When the experiment was repeated on cow no. 9, the results on the Reichert-Meissl and Polenske values were not duplicated. Instead; the Reichert-Meissl and Polenske values decreased. Similar results were obtained on cow no. 10, which received 975 g. of butyrate and 1092 g. of acetate over a period of 22 hours. Cow no. 11 received a total of 786 g. of butyrate over a period of 34 hours, during which time the animal was on full feed. The butyric acid did not alter the fat constants from the normal values.

Cow no. 12, which had been on a regime identical to that of cows 9 and 10, was fasted completely for 35 hours. The decrease in the Reichert-Meissl value was similar to that observed in cows 9 and 10. The Polenske value showed a more marked change, however, decreasing from 2.82 to 0.92, whereas the fasted cows receiving butyrate decreased from 3.35 to 2.17 in one case and from 2.81 to 2.14 in the other.

DISCUSSION

With neither blood lactic acid nor blood pyruvic acid being utilized in measurable quantities by the active mammary gland of the normal cow (8, 12), the only possible blood carbohydrate which could supply sufficient carbon to account for the lower fatty acids of milk fat is that of blood glucose. The experiments described herein show that when a continuous flow of glucose is administered intravenously at the highest possible rate tolerated by the cow for a period in excess of 2 days, the decrease in the lower fatty acids of the milk fat caused by inanition is not prevented. It must be concluded that blood carbohydrate *per se* is not the precursor of the lower fatty acids of milk fat.

It appears clear that carbohydrate administered orally is not a direct precursor of the lower acids (7), but probably exerts a sparing effect or is converted into the necessary precursors in the alimentary tract.

It is known that the active ammmary gland produces urea (4) and contains arginase (14) which may be used by the gland for the deamination of amino acids. The quantities of amino acids taken up by the gland are sufficient to account for the carbon in the lower fatty acids. This appeared to be a possibility when it was shown that the active gland of the fasted ruminant did not take up the free amino acids from the blood (10, 16). However, the failure of an intravenous administration of a protein hydrolysate to prevent the decrease in the lower fatty acids of the milk of the fasted cow appears to rule out this possibility.

Sodium oleate was fed by the constant flow method to test the earlier hypothesis of Hilditch and Paul (6) and of Shaw and Petersen (15) that the lower fatty acids are derived from the degradation of the longer chain fatty acids. The results do not indicate that the lower acids are derived from oleic acid. However, the amount of oleate injected was necessarily small and definite conclusions probably are not warranted.

In view of the recent work showing that acetic acid is used in the synthesis of fatty acids, this substance appeared to offer considerable promise as a precursor of the lower fatty acids of milk fat, since it is formed in the rumen in considerable quantities. Further, such synthesis would explain the ability of food carbohydrate but not blood carbohydrate to serve as the precursor of the lower acids. However, the Reichert-Meissl and Polenske values on milk fat from fasted ruminants receiving considerable acetic acid abomasally and intravenously were typical of fasted animals.

Butyric acid, being produced in the rumen by the fermentation of carbohydrate, appeared to be another possible precursor substance. Four cows received relatively large quantities by the intravenous route. The results are conflicting. In one case there was actually an increase in the Polenske value, and the normal Reichert-Meissl value was maintained even though the cow was fasted. These results were not duplicated in succeeding experiments, although the Polenske value of the milk fat of the two additional fasted cows receiving butyrate did not decrease nearly so much as in the case of a fasted control cow. While no conclusions appear to be warranted, the data do suggest some interesting possibilities.

It may be that the butyric acid was being converted to acetone bodies and burned at such a rapid rate that little reached the mammary gland except in the one case. The same could also apply to acetic acid. On the other hand, if butyric acid was being made available to the gland in these experiments and was not being incorporated into the triglyceride, it will necessitate thinking in terms of something more complicated than a simple recombination of glycerol and fatty acids in the formation of the triglycerides of milk fat.

There has been considerable disagreement as to whether blood carbohydrate is the precursor of the lower fatty acids of milk fat. Because of the considerable amount of data which has been accumulated in the past few years in studies concerned with this possible relationship, and because of the rather conclusive negative data reported in this paper, it may be well to examine and summarize the data collected to date.

The suggestion that the lower fatty acids of milk fat are synthesized directly from carbohydrate is based entirely upon the reports that the respiratory quotient of the normal active gland of the ruminant exceeds unity (3, 9, 12) and that the respiratory quotient of the gland of the fasted ruminant is less than unity (9, 16). It is pointed out that these two findings coincide with the fact that fasting decreases the short-chain fatty acids as well as the carbohydrate content of the body. The high respiratory quotient (RQ) of the active normal gland, which was first reported by Graham *et al.* (3), appears to be established fairly well. The reports by Reinecke *et al.* (9) and by Shaw *et al.* (16) that the RQ of the gland of the fasted ruminant is less than unity, are based on only a few observations.

Because of the difficulty involved in obtaining representative RQ's on the gland, many more data are needed to establish the latter with certainty. In addition, Reinecke *et al.* reported that 48 hours or more of fasting were required to produce a low RQ. It is difficult to correlate this with the fact that the greatest decrease in the lower fatty acids occurs within the first 24 hours of fasting. However, assuming that the RQ of the gland of the fasted ruminant is low, what does it represent in terms of the metabolism of the gland?

In the first place, most investigators have been unwilling to accept the RQ alone on any particular organ as proof of the type of metabolism taking place within that organ. Recent work showing that carbon dioxide actually is used in synthesis within the body has tended to confirm this view. Secondly, there is considerable doubt of a true metabolic relationship between the decrease in the lower fatty acids and fasting on the one hand, and the lowering of the RQ of the gland reported for the fasted ruminant on the other, since it has not been possible to demonstrate a consistently low RQ for the glands of cows in which the lower acids had been decreased markedly by the feeding of cod-liver oil (12).

Even if it were possible to establish such a relationship, it must be kept in mind that neither the feeding of cod-liver oil nor fasting decreases the lower acids more than 35 to 40 per cent. If the lower fatty acids are synthesized from carbohydrate, how then can we account for a low RQ with 60 per cent or so of these acids still being so synthesized?

The amount of glucose available to the gland for the synthesis of fat appears to be entirely insufficient for the synthesis of the lower fatty acids. Graham first attempted to measure the blood flow in relation to glucose uptake by the gland, using the thermostromuhr method, and concluded that the glucose uptake was insufficient to account for more than 50 per cent of the milk lactose (2). At the time it was believed that the thermostromuhr method of measuring blood flow in vivo was quite accurate. However, very exhaustive experiments by Gregg *et al.* (5), Shipley *et al.* (18), and Shipley and Gregg (17) have shown that such is not the case. They obtained errors as great as 300 per cent and stated that " . . . the flow of blood in an artery of an animal can only by chance happening be determined from a unit applied to it which had been previously calibrated in an artificial circulation system . . . this is so, because, for the same unit it is indeed an accident when in vitro and in vivo environments influence the differential temperature-flow relations in the same direction and to the same extent."

By determining the calcium, inorganic phosphorus, and glucose uptake by the gland and the total amount of calcium, phosphorus, and lactose secreted during a 24-hour period, it was possible to calculate the blood flow, the total amount of glucose utilized by the gland, and the amount of glucose available for other purposes after accounting for lactose (16). Five such experiments showed a blood flow of 494 volumes for each volume of milk formed, with a range from 408 to 561. The glucose uptake was sufficient to account for 105.5 per cent of the milk lactose. In the five experiments it was found that the glucose uptake was sufficient to account for 81.4, 101.2, 115.4, 115.3, and 97.9 per cent of the lactose secreted.

As any mammary gland balance must depend upon the accuracy of the arteriovenous determinations in representing the true metabolism of the gland, this method of approach is subject to a great deal less error than the thermostromuhr method. The accuracy of a mammary gland balance based on the thermostromuhr method is dependent upon whether the arteriovenous differences represent the true average uptake by the gland as well as the accuracy of the determination of the rate of blood flow. The large errors reported for the latter method are much greater than that which would be expected where the rate of blood flow is calculated from the uptake of calcium and phosphorus and the total amount of calcium and phosphorus secreted in the milk as shown by the actual analysis of the milk.

Data obtained by Shaw and Petersen (15) and Shaw, Boyd, and Petersen (13) can be used to make similar calculations, although in this case the average analysis of milk must be used, and the blood calcium and glucose uptake do not necessarily represent the same cow or experiment. However, the values are sufficiently numerous and uniform to obviate gross errors. Twenty arteriovenous differences for plasma calcium averaged 0.31 mg. per cent. Converting this to whole blood values on the basis of a 30 per cent cell volume, the difference becomes 0.217 mg. per cent. Assuming that the average calcium content of the milk is 120 mg. per cent, the ratio of calcium in the milk to calcium uptake becomes 553 to 1. The average of 40 determinations of the glucose utilization by the gland was 9.3 mg. per cent. At a ratio of 553 volumes of blood to one unit volume of milk, the glucose uptake could account for 5142 mg. per cent of lactose. With an average lactose content of 4900 mg. per cent in milk, this represents sufficient glucose to account for approximately 105 per cent of the lactose. The five complete balances of Shaw *et al.* (16) are in good agreement with these calculations. The complete balance experiments included fat. To account for the lower fatty acids in these trials, the data show that from 25 to 30 per cent of the glucose would be needed if we are to postulate that the lower fatty acids are formed from carbohydrate. Even with the small amount of glucose taken up as glyco-protein (10), it appears obvious that with neither blood lactic acid nor pyruvic acid being utilized by the gland, the gland does not remove sufficient carbohydrate from the blood to account for both lactose and the lower fatty acids.

Also opposed to the suggestion that fasting causes a decrease in the lower acids by depleting the carbohydrate available for the synthesis of these acids, is the finding that the gland of the cow with ketosis continues to remove a normal amount of glucose from the blood even when the blood glucose falls as much as 50 per cent (11), a value much lower than would be attained by two days of fasting. Further, it was observed that even such low values were not associated with a decrease in the lower fatty acids in those cases of ketosis in which the animals retained their appetites, indicating a relationship between feed intake and the lower acids, but not between blood glucose and these acids.

Finally, with the data reported in this paper to the effect that the maintenance of the blood glucose of the fasted cow at a high level does not prevent or retard the decrease in the lower fatty acids, the evidence against the postulation that these fatty acids are synthesized in the gland from carbohydrate appears to be conclusive.

SUMMARY AND CONCLUSIONS

1. A technic was developed for the continuous intravenous feeding of ruminants.
2. The continuous intravenous injection of cows with a protein hydrolysate, glucose, oleic acid, and acetic acid failed to prevent the decrease in the lower fatty acids of milk fat caused by fasting. As much as 1984 g. of sodium acetate was administered in 22 hours and as much as 1985 g. of glucose was administered in 31 hours.
3. A summary of the work to date renders the theory of a blood carbohydrate origin of the lower fatty acids extremely unlikely.
4. The data obtained from the continuous injection of butyric acid are inconclusive.

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THE REMOVAL OF THE SORBED GASES IN DRIED MILKS[1]

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INTRODUCTION

The results of experiments initiated in 1942 (2) on dried milks packed in air and in inert gas indicated that the keeping quality of the products packed in an inert gas atmosphere of which the final oxygen concentration was 3 to 4 per cent was considerably greater than that of the products packed in air (20.9 per cent oxygen). Although the keeping quality of dried milk depends to a great extent on the freshness of the liquid milk used and the methods employed in its desiccation, the improvement in keeping quality that was effected by reducing the oxygen concentration of the containers was so marked that recommendations were made for the consideration of this method of packing for all dried milks which had to undergo rigorous conditions of storage, especially those destined for overseas use (4). Reduction of the oxygen concentration in the commercial containers to values of less than 3 per cent did not seem practical at that time. The results of Lea, Moran, and Smith (3) published in 1943 indicated also that the keeping quality of dried milks was increased when the oxygen concentration within the dried milk containers was decreased. Subsequent studies (1, 5, 6) confirmed these observations and indicated the methods necessary to obtain oxygen concentrations of different values.

Although the practice of packaging dried milk in atmospheres of reduced oxygen concentration had been used to a limited extent for some time in the industry when this work was begun, information was lacking on the amounts of gases held in dried milks by various forces, the factors that are concerned in the removal of these gases, and the relative effect of different oxygen concentrations on keeping quality.

Spray-dried milks are composed of finely divided and very porous particles and, therefore, have enormous total surface areas (internal as well as external) per unit weight of product. Thus it seems that gases may be held by the particles by adsorption and by occlusion, and in the fat by absorption. In the following discussion these gases, however held, will be considered together under the designation of sorbed gases.

Aside from the specific characteristics of a porous material, the temperature, time, and degree of evacuation are important factors in the removal of

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sorbed gases. Hence, the effect of variations in these factors, as well as of the moisture content, on the amount of sorbed gases was studied to determine the relative importance of each factor and to determine the practical conditions that may be employed to remove these gases from dried milks.

APPARATUS AND ITS OPERATION

A diagram of the apparatus used throughout these studies to remove the sorbed gases, measure their volume, and determine their composition is shown in figure 1.

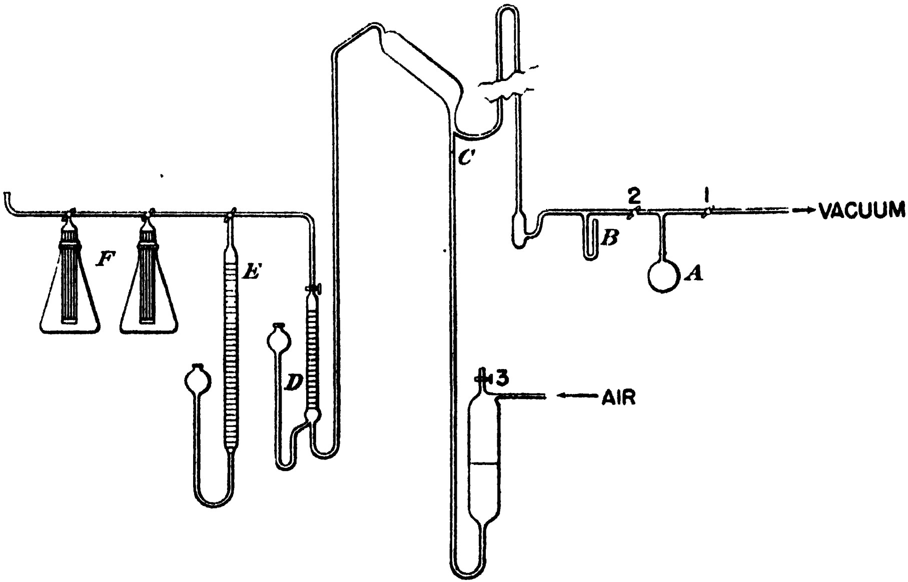

FIG. 1. Toepler pump and gas analysis system.

A is a 500-milliliter ground-glass-joint flask that may be immersed in a constant-temperature bath. *B* is a mercury manometer, *C* a Toepler pump, *D* the gas burette, *E* the gas-collecting chamber of the gas analysis apparatus, and *F* the absorption system, consisting of a carbon-dioxide absorption burette containing potassium hydroxide and an oxygen-absorption burette containing alkaline pyrogallol solution. The manifold system is made of capillary tubing and the horizontal portion is mercury filled. The absorption burettes and the gas burette are connected to the manifold by three-way T stopcocks.

The flask *A* containing the 225 g. sample was immersed in a bath held at the desired temperature. The oil vacuum pump was then operated and the sample evacuated for the desired period of time at the desired degree of vacuum. When the vacuum required was less than the full vacuum of the pump, it was obtained by adjusting stopcock 1 and observing the manometer *B*, stopcock 2 being open to the Toepler system. After the desired vacuum treatment, stopcocks 1 and 2 were closed and the Toepler system completely evacuated by operating stopcock 3. Stopcock 2 then was opened and the residual sorbed gas in the sample was desorbed over a period of about 6 hours by heating the sample in *A* to 70° C. (or higher) and operating the Toepler pump at intervals of about 30 minutes to remove the gas and store it in burette *D*, where it was measured. When it was desired to analyze the desorbed gas, it was transferred to burette *E*, where it was then measured both before and after absorption of the carbon dioxide and the oxygen in the appropriate solutions.

EXPERIMENTAL

In the following discussion the term "residual gas" has been used to designate the gases which can be removed from the system as indicated above after a given evacuation procedure has been used. The total amount of residual gases consists of the free gases remaining in the system plus the sorbed gases which can be removed from the product. After an evacuation at a vacuum of 3 to 5 mm. pressure, the amount of free gas in the container is but a small proportion of the total amount of residual gas. The proportion increases as the degree of evacuation decreases.

The amount of residual gas in a container varies with the efficiency of the evacuation process, *i.e.*, degree, temperature, and time of evacuation. The amount of residual gas which can be removed with a Toepler pump varies with the temperature at which the product is held, the time of evacuation, and the physical structure of the product.

At a given temperature the amount of residual gas obtained with a Toepler pump during the first interval of time is relatively large and decreases with each successive interval. After 6 hours the amount obtained at each interval is very small and practically a constant. The total amount obtained during 6 hours was considered the residual gas content of the product.

If the temperature of the product during desorption is increased, the amount of residual gas increases as indicated in figure 2.

At temperatures greater than 70° C., although larger volumes of gas are obtained, slight discoloration of the product occurs, suggesting slight decomposition. A temperature of 70° C. was therefore chosen as that at which desorptions were to be carried out.

Although the total amount of sorbed gases was not removed under these conditions, the values obtained are comparable. From data obtained it was estimated that approximately 80 per cent of the residual gas was removed in the manner prescribed, at 70° C.

The Effect of the Temperature of the Product during Evacuation upon the Amount of Residual Gas

An increase in the temperature usually decreases the amount of gas adsorbed by a porous material. Samples of dried milk were treated at a vacuum of 10 mm. pressure for 10 minutes at temperatures of 20°, 30°, and 40° C., respectively, and the amount of residaul gas then determined as previously described. The amounts of residual gas obtained at 30° and 40° C. were slightly but not significantly less in each case than the amount obtained at 20° C. However, when short periods of evacuation and products of moisture contents greater than usual are concerned, the efficiency of evacuation will be affected by the vapor pressure of the product, this pressure increasing with temperature. Under these conditions the efficiency of the process may be less at the higher temperatures. One-half-pound samples of a commercial dried milk were subjected to the vacuum of an oil pump for 3 minutes at 20°, 30°, 40°, and 50° C., respectively, and the actual vacuum attained in the system was noted. The values are shown in figure 3. Under similar conditions of evacuation, a number of tins of dried milk were packaged in nitrogen. After storage of 3 days at room temperature, the gases in the head space of the cans were analyzed. The average oxygen percentages of the gas in the cans evacuated at the different temperatures are shown in figure 4. It is evident that the values obtained are those which can be obtained only under the conditions used. They will differ with the moisture content of the product, the time of evacuation, the capacity of the pump used, and other factors. However, the results do emphasize the fact that

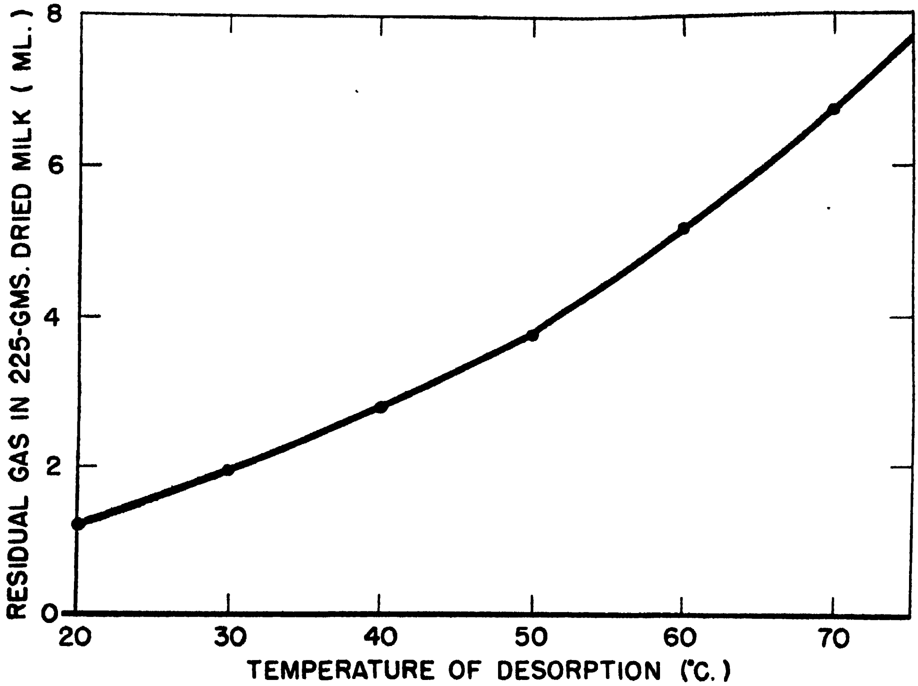

FIG. 2. Amounts of gas obtained at different temperatures of desorption.

increasing the temperature at which the product is held does not necessarily increase the amount of gas removed by evacuation.

The Effect of the Time of Evacuation upon the Amount of Residual Gas

It is logical to assume that the longer a dried milk is held under a vacuum the less will be its residual gas content.

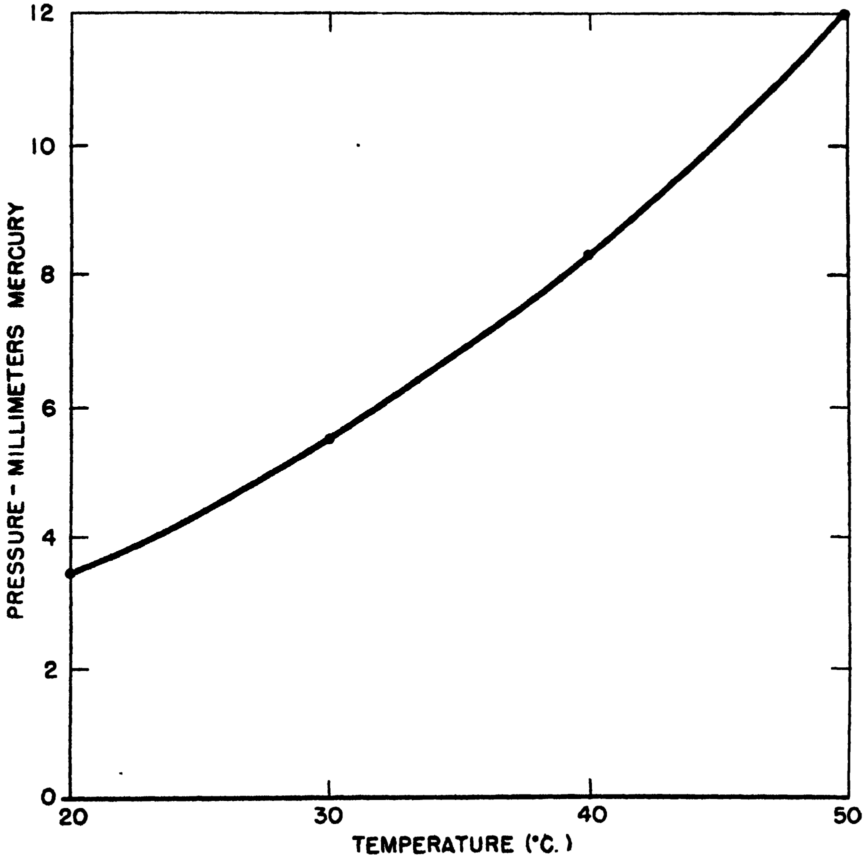

FIG. 3. Manometric pressure obtained by evacuation of dried milk for 3 minutes at different temperatures.

Figure 5 shows the results on an old sample of dried milk after treatment at various degrees of vacuum at room temperature. The values shown indicate that from 15 to 30 minutes is required to evacuate dried milks to a point where additional evacuation will produce only a small regular decrease with increase in pumping time. As expected, the amount removed increases with the time of evacuation, and increases more at the higher degrees of evacuation.

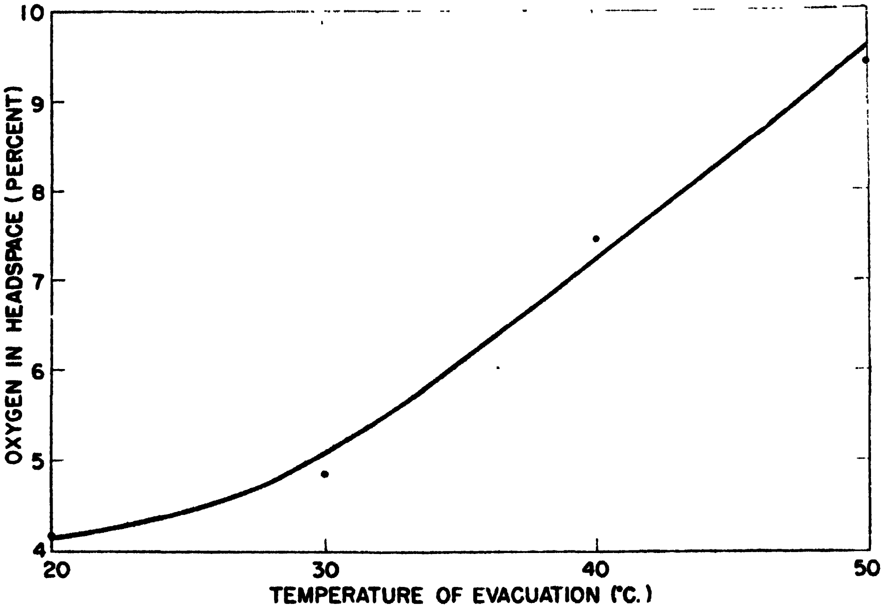

Fig. 4. Oxygen percentage in dried milk containers evacuated for 3 minutes at various temperatures.

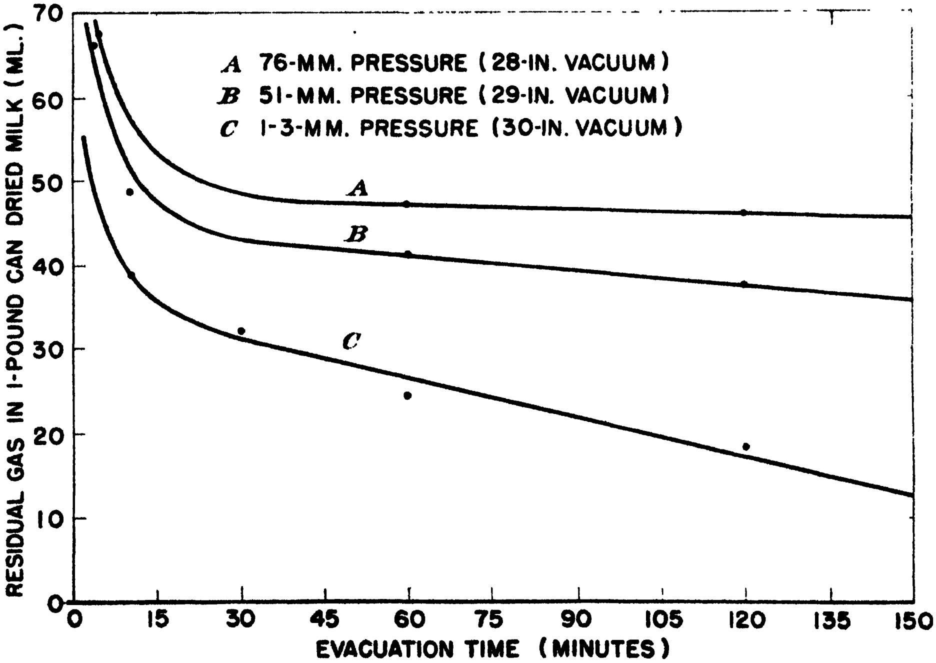

Fig. 5. Amounts of residual gas obtained from dried milks evacuated at different degrees of vacuum for different periods of time.

Effect of the Degree of Vacuum Used upon the Amount of Residual Gas

The time of evacuation used in these experiments was 10 minutes, the temperature 25° C., and the degree of vacuum 10 mm. pressure of mercury. The results are given in figure 6.

It is apparent from these data that the amount of sorbed gas decreases but slightly as the degree of vacuum increases from 51 to 3 mm. of pressure. The amounts of gas removed (*B*) at 25° C. represent the gas in the free space of the container after evacuation under the given conditions. The values represented by *A* are the amounts of sorbed gas obtained when the temperature was increased to 70° C. The total amounts of residual gas are represented by *A* + *B*.

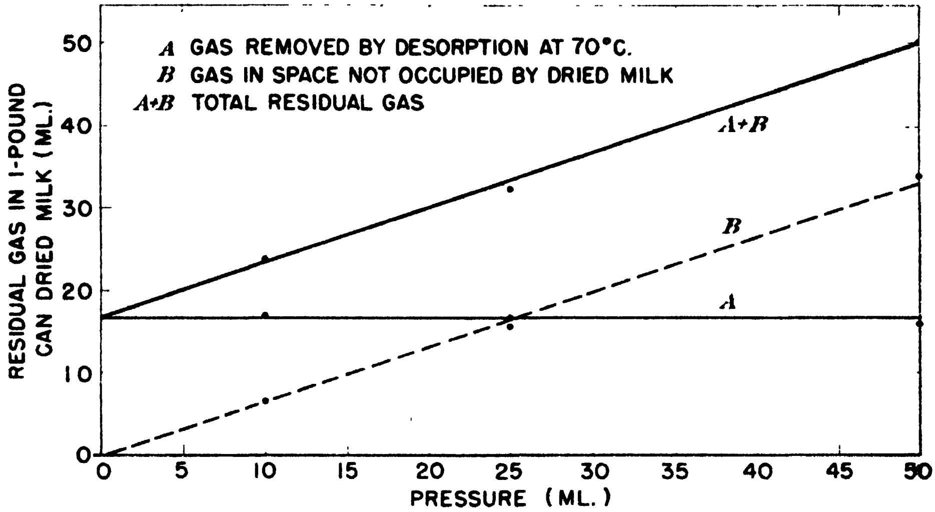

FIG. 6. Amounts of residual gas in dried milks evacuated at different degrees of vacuum.

From the data presented it is evident that increases in time, temperature, and pressure of evacuation beyond practical values do not decrease greatly the amount of the sorbed gases held by the product. However, the desirability of the use of as complete a vacuum as possible is indicated in figure 6. At vacuums of several millimeters pressure, the amount of gas in the free space is, of course, very small. At 25 mm. of pressure it is approximately 17.50 ml. or 3.65 per cent of the free space in a 1-lb. container and is approximately equal to the amount of residual gas in the dried milk which can be removed by heating the product to 70° C. Upon evacuation at a vacuum of 51 mm. pressure, the amount of gas in the free space is approximately twice that of the residual gas.

Effect of the Concentration of the Milk upon the Residual Gas of Its Dried Product

It had been noted in preliminary experiments that dried milks made by different methods and of different degrees of fineness differed greatly in the amounts of residual sorbed gases. Samples of dried milk therefore were prepared from milks of different degrees of concentration varying from

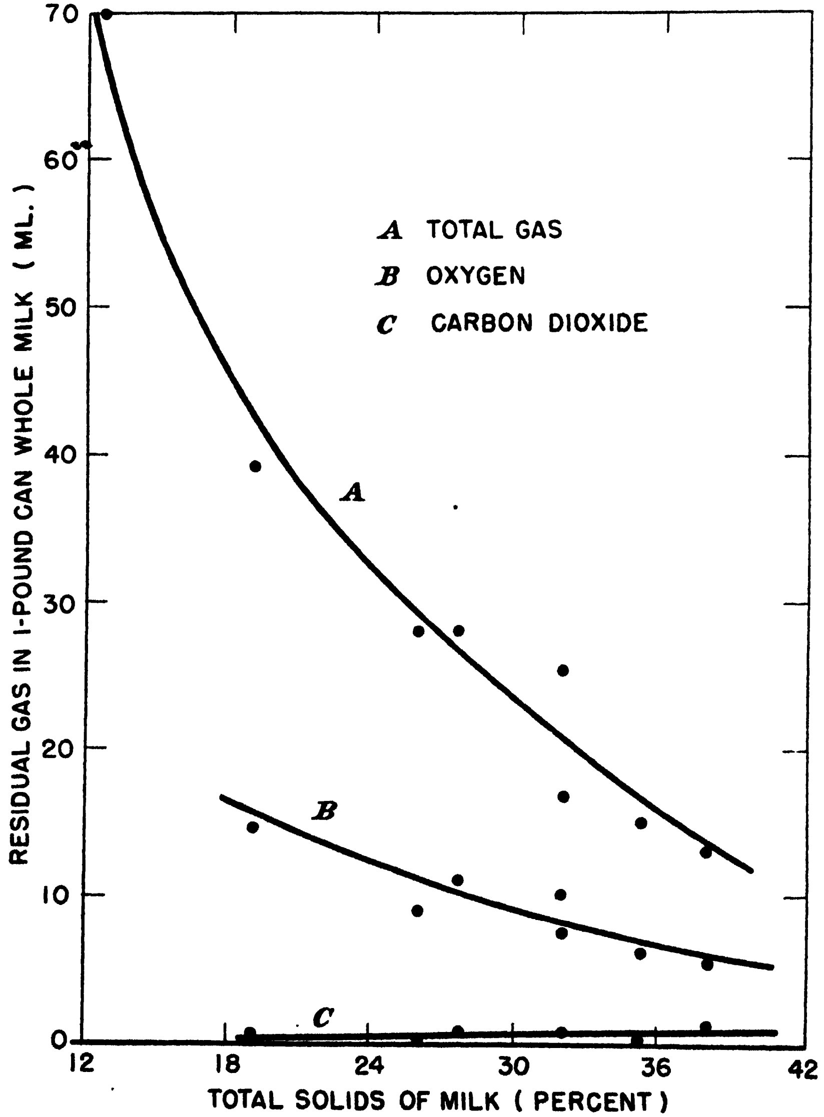

FIG. 7. Residual gas in dried milks prepared from milks of different concentration.

13 per cent to 38 per cent solids. The product made from milk of 13 per cent solids was finely divided and of low apparent density. As the concentration of the milk increased, the dried milks became coarser in texture and of greater apparent density. The results on determinations of residual gases in these samples are given in figure 7.

The amounts of residual gas, or gas which remained in the products after treatment at full vacuum of an oil pump for 1 hour at 25° C., decreased rapidly with increases in the solids concentration of the milk used. The decrease in the amounts of oxygen was relatively smaller.

Effect of Moisture Content upon the Amount of Sorbed Gases

A series of experiments was conducted to determine the effect of variations in the moisture content on the amount of sorbed gases remaining in dried milks after vacuum treatment.[7] Samples of two dried milks containing less than 2 per cent of moisture were allowed to absorb moisture from relatively humid atmospheres until the desired moisture content was obtained

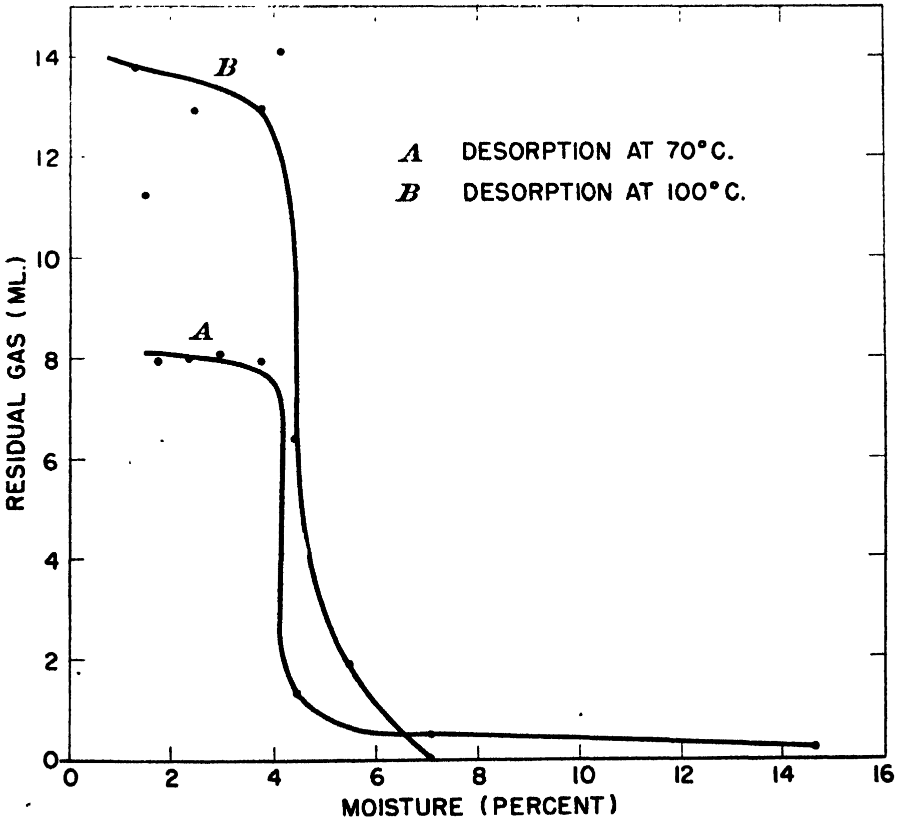

Fig. 8. The residual gas in dried milks of different moisture percentage.

in each case. Samples ranging in moisture content from the original amount to over 7 per cent were obtained in this manner. Each sample was subjected in turn to the full vacuum of an oil pump for 1 hour at 25° C., with an anhydrous magnesium perchlorate desiccant inserted between the sample and the remainder of the system. The residual gas of each sample was then determined with the temperature of the sample maintained at 70° C. The results are shown in figure 8.

Within the range of moisture content of commercial dried milks the amount of sorbed gases differed but slightly with differences in the moisture content. However, if the products absorbed moisture to amounts greater than 4 per cent, there was an abrupt decrease in the amount of sorbed gases. Whether the absorption of moisture facilitates in some manner the removal of gases during evacuation or whether the moisture is adsorbed preferentially has not been determined.

Diffusion of Sorbed Gases

To what extent the sorbed gases can be removed by the removal of nitrogen into which these gases have been allowed to diffuse for a period of time was determined.

TABLE 1

Percentage composition of gas in container after successive evacuations, filling with nitrogen, and storing for different periods of time

Period	Nitrogen	Oxygen	Carbon dioxide
	(%)	(%)	(%)
1st (3 days)	97.8	2.2	Trace
2nd (3 days)	99.6	0.4	Trace
3rd (4 days)	99.8	0.2	Trace
4th (3 days)	100.0	Trace	Trace
5th (4 days)	100.0	Trace	Trace

A freshly made sample of 225 g. of dried milk quickly was brought to full vacuum with the oil and Toepler pumps. Nitrogen then was bled into the system until the system was brought to atmospheric pressure; the sample was allowed to remain under these conditions for 3 days. A sample of the gas in the system then was withdrawn and analyzed, and the system again evacuated and filled with nitrogen. This cycle of procedure was repeated until the percentage of oxygen content of the nitrogen admitted was practically zero. The results are shown in table 1.

After five stages of evacuation the system containing the sample was evacuated quickly again with the oil and Toepler pumps to rid it completely of free gases. The sample container then was immersed in a bath maintained at 70° C. and the liberated gases were removed with a Toepler pump and analyzed.

The amount obtained was 6.15 ml., which is equivalent to 70.1 per cent of the amount of sorbed gases that were removed from another sample of the same milk under similar conditions, except that no diffusion was allowed to occur. The composition of the residual gas obtained after the five successive evacuation and diffusion periods was as follows: Nitrogen, 95.9 per cent; oxygen, 4.1 per cent; carbon dioxide, trace. Hence, it is indicated that although all of the oxygen cannot be removed except by continued exhaustive removal by desorption and evacuation extending over a long period of time, the amount of oxygen which remains in the residual gas after two cycles of this process is relatively small.

Percentage Composition of the Residual Gas

Aside from the amounts of gas retained by the dried milks after evacuation, the oxygen concentration in this gas is of interest. In table 2 are shown the results of analyses of the residual gases from two freshly made and three relatively old samples of dried milk.

TABLE 2

Composition of residual gases from representative samples of dried milk

No.	Description of sample	Oxygen	Nitrogen	Carbon dioxide
		(%)	(%)	(%)
1	Freshly made. Evac. 29.6 in., 10 min., at 25° C.	32.7	68.9	1.4
2	Freshly made. Evac. 29.6 in., 15 min., at 25° C.	39.0	61.0	0.0
3	Old sample. Evac. 30 in., 5 min., at 25° C.	25.6	65.0	9.4
4	Same as no. 3. Evac. 30 in., 10 min., at 25° C.	22.0	68.9	9.1
5	Same as no. 3. Evac. 30 in., 45 min., at 25° C.	23.7	66.1	10.2

The values in table 2 indicate that the amount of residual oxygen is relatively high and that of carbon dioxide is practically nil in freshly made dried milk. In aged samples the proportion of oxygen is less and the carbon dioxide considerably more than in freshly made dried milks. Also, the proportion of carbon dioxide in the residual gas is considerably more than would be expected if only the partial pressures of this gas in the atmosphere were concerned. However, carbon dioxide is adsorbed more strongly than the other gases concerned and therefore seems to concentrate in the product with a resulting displacement of other gases, in this case evidently oxygen.

These results support the belief that adsorption is a vital factor in the retention of gases by dried milks. If occlusion alone were concerned, the percentage composition of the gases would be that of the air used in the drying procedure and also would not vary greatly with the age of the product. The release of oxygen by the product with age is of interest. To what extent it can enter into consideration in practical procedures requires further study.

DISCUSSION AND SUMMARY

The amount of sorbed (occluded, adsorbed, and dissolved) gases in dried milks varies greatly with the fineness of the product. Dried milks made from milks of normal concentration contain a relatively large amount of sorbed gas, which decreases greatly as the concentration of the milk used is increased from 9 to 38 per cent solids.

A large percentage of the sorbed gases can be removed by evacuation for relatively short periods of time within the practical range of temperatures of 20° to 40° C. The remainder only can be desorbed very slowly as the time of evacuation is extended.

The composition of the sorbed gas varies with the storage time of the dried milk in an atmosphere of air. Freshly made products seem to have percentage concentrations of oxygen greater than air and very low percentage concentrations of carbon dioxide. In older products, the proportion of oxygen in the sorbed gases is but slightly greater than that in air and the proportion of carbon dioxide is greater than can be accounted for if the gases are occluded air.

The results indicate that most of the residual gases are held by adsorption forces.

A large proportion of the oxygen of the sorbed gas may be removed by successive evacuations, with periods of several days between to allow for diffusion of the oxygen into a nitrogen atmosphere. The results indicate that two cycles of evacuation—filling with nitrogen, and holding for 3 to 4 days—remove a high percentage of the oxygen of the sorbed gases.

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ASSOCIATION ANNOUNCEMENTS

The Annual Meeting of the American Dairy Science Association will be held at Ontario Agricultural College, Guelph, Ontario, June 24, 25, and 26, 1947. Further information concerning the meetings will appear in later issues of the JOURNAL.

D. M. Seath, chairman of the Production Section, has appointed the following men to the Committee on Dairy Cattle Judging Contests: S. M. Salisbury, Ohio, *Chairman;* D. L. Fourt, Idaho; and R. E. Johnson, Connecticut.

The membership of the Honors Committee, as listed on page 66 of the January issue of the JOURNAL, was incorrect. The members of this committee are: A. C. Dahlberg, New York, *Chairman;* A. C. Ragsdale, Missouri; and J. A. Nelson, Montana.

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THE ACID DEGREE, FREE VOLATILE FATTY ACIDS, AND THE FLAVOR SCORE OF SALTED COMMERCIAL BUTTERS[1]

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It is well known that butter deteriorates during long storage as a result of oxidative or hydrolytic processes with a subsequent formation of various chemical substances. The extent of the deterioration is governed by such factors as the past history of the butter and the conditions of storage. A number of chemical changes occur in butter as a result of storage. An interesting example of these changes is found in the work of Browne (2) who noted that a sample of butter exposed to the air at room temperature for a period of about 25 years markedly increased in the content of free acids and total volatile acids and decreased in the unsaturated insoluble acid content.

Various attempts have been made to use the acid degree and the free volatile acidity of butterfat as a means of following the quality change in butter. Ferris, Redfield, and North (3) found that after keeping sweet cream butter in cold storage 5 to 6 months there was a lowering in the score of one point, while the free volatile fatty acid value was about doubled. After 6 to 7 months the butter was removed from cold storage and kept at 15° C. for 2 weeks, causing a drop in score but no increase in volatile acids. Bendixen (1), working with the acid number of fresh butter before and after storage, noted that an increase in the acid ratio (fat acidity: butter acidity) during a week at 21° C. and during 1 month at 0 to 5° C. seemed to be closely related to poor keeping quality, especially in the case of sweet cream butter. In an extensive study dealing with butter, Fouts (4) reported that most samples of unsalted butter increased in acid number of the fat during holding for 6 days at 21° C.

The relationship, however, between the acid degree, free volatile acidity, and the quality of commercial butters covering the normal flavor score has never been clearly established. Fouts (4), using butter obtained for the most part from a student scoring contest and generally scoring 90 or over,

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could find little or no correlation between the acid degree and score of salted commercial butter. On the other hand, Knaysi and Guthrie (11) developed a colorimetric method for determining quality of commercial butter based on the principle that an indirect relationship existed between quality of commercial butters and concentration of free fatty acids. Guthrie (8) later stated that values obtained on a large number of butters by this method and by the method involving titration of the fatty acids correlated closely with each other, and both agreed with the flavor scores in the great majority of cases.

The possible existence of a relationship between free volatile fatty acids and the quality of commercial butter apparently requires further investigation. An attempt by Fouts (6) to study the relationship between degree of rancidity and volatile fatty acidity was limited to 14 samples of unsalted butter, all of which were rancid to some degree. It was concluded that no correlation existed between degree of rancidity and volatile fatty acid concentration.

It would appear that if a relationship exists between fat acidity and quality of experimental butter kept in storage, as indicated by Bendixen (1), a similar relationship, possibly modified to some degree, might be shown to exist with commercial market butter.

Most of the market butter today is made from pasteurized cream. As the pasteurization process inactivates lipolytic enzymes of milk and cream, there is much less chance that hydrolytic rancidity will occur. However, poor quality butters, even if manufactured from pasteurized cream, may have a high free fatty acid content as a result of certain factors. The cream prior to pasteurization may have developed some rancidity. Although some of the volatile fatty acid contributing to this rancidity would be washed out of the butter, Ferris, Redfield, and North (3) have shown that more remained in the butter from cream of high volatile acidity than in butter from cream showing low volatile acidity. In a similar way, Jack, Tarassuk, and Scaramalla (10) have obtained data which show that as the acid degree increased the flavor score of butter made from rancid cream decreased. Certain microorganisms can hydrolyze butter-fat. If the cream is improperly pasteurized or if recontamination after pasteurization occurs, these organisms may be present and eventually increase the free fatty acid content of the butter. Finally, the production of free fatty acids through oxidative rancidity should be considered.

As there was on hand a new method considered sensitive enough to determine the free volatile fatty acid content of butter over the normal marketable flavor score, a study was undertaken to investigate the possible relationship between free volatile fatty acid concentration and flavor score of salted commercial butters. At the same time, determinations of acid degree and Knaysi-Guthrie number (11) were conducted on the same butters to see to what degree these constants were interrelated with each other and with flavor scores of salted commercial butter.

EXPERIMENTAL METHODS

Butter samples for this study were obtained in 1-lb. lots, either directly from grocery stores throughout the central New York State area or from various plants manufacturing butter throughout the state. At first the plan was to obtain all samples of butter from different groceries, but due to extreme shortages less than half of the samples could be obtained in this manner. The buttermaking concerns were instructed to send a variety of grades of marketable butter which was held by them in storage. A total of 69 samples of salted butter was obtained.

As the samples of butter were received they were placed in a cold storage room until ready to be tested, which was usually 2 or 3 days later. To prepare the butters for analytical testing, each sample had its outer layer (0.25 inch thick) removed and then some of the interior portion was melted to a liquid state in a water bath having a maximum temperature of 50° C. The melted butter was next centrifuged for a few minutes and the oil layer drawn off by means of a suction pump into a clean flask. The serum also was saved for analyses.

Analytical methods used in this work included the following:

Acid degree. The total fat acidity was determined by the recently published method of Herrington and Krukovsky (9). In this method 5 g. of butter oil were placed in a small flask, followed by the addition of 20 ml. neutral alcohol and 5 drops of 1 per cent phenolphthalein indicator (1 per cent in 50 per cent alcohol solution). The solution was heated to boiling and titrated with N/20 NaOH in 50 per cent alcohol. The results were reported as ml. N alkali required to neutralize 100 g. of fat. Acid degree and acid number were considered synonymous in this work.

Knaysi-Guthrie number. This technic gives an index of free fatty acids. The method was applied to butter by Knaysi and Guthrie (11) to estimate quality. To 1 ml. of clear butter oil in a test tube, 3 ml. of neutral red dye (pure xylol saturated with the base of neutral red) were added and the mixture well agitated. The tube was compared to a set of color standards containing known quantities of oleic acid.

Free volatile fatty acids in butter oil. Twenty grams of melted butter oil were mixed with 175 ml. of ethyl ether and the whole placed in a separatory funnel. The ether-fat solution was next washed six times with N/10 NaOH in a manner described by Gould and Johnson (7). The alkali washings carefully were heated over a hot plate to remove any ether; then they were placed in a Kjeldahl flask (800 ml.) to which 35 g. of $MgSO_4 \cdot 7\,H_2O$ and a few glass beads already had been added. The mixture was brought to pH 2 with 50 per cent H_2SO_4, refluxed for 5 minutes, and distilled in a slightly modified Kjeldahl distillation apparatus recently described by Smiley, Kosikowsky, and Dahlberg (12). Distillation continued until crystallization occurred. The distillate and neutral alcohol rinse of the condenser tube were titrated with N/20 NaOH. Both water-soluble and water-insoluble volatile fatty acid values were recorded.

Free volatile fatty acids in butter serum. The recovery of the volatile acids in the serum was conducted as follows: Ten grams of serum were added directly to a Kjeldahl flask containing 35 g. of $MgSO_4 \cdot 7H_2O$, a few glass beads, and 280 ml. of distilled water. Enough 50 per cent H_2SO_4 was added to lower the pH of the mixture to 2. After refluxing for 5 minutes and rinsing down the refluxer with 15 ml. of distilled H_2O, the mixture was distilled. The distillate (285 ml.) with the neutral alcohol rinsings from the distillation apparatus was titrated with N/20 alkali. Water-soluble and water-insoluble acids were measured.

Total free volatile fatty acids in butter. In order to arrive at the total free volatile fatty acid content of the butter, it was assumed that for all butters the serum constituted one fifth of the butter. On this basis, the total free volatile fatty acidity of the butter was calculated on a ratio of four parts butter oil to one part butter serum. In routine analysis the tests would be made on the butter oil and serum from a weighed sample of butter.

Butter score. The butter was graded by two of the authors. Butters were scored for flavor only, but were marked on the basis of 93 to < 85 or from excellent to very poor. Butters scoring lower than 85 were considered to be unmarketable.

EXPERIMENTAL RESULTS

Since the acid degree and the Knaysi-Guthrie number deal only with butter oil, it was necessary for comparative purposes to obtain the total and water-soluble volatile fatty acids of the butter oil. Furthermore, since it was felt that the key to certain relationships might be associated with the volatile acids of the serum, this portion of the butter also was analyzed. The results obtained from the oil and serum made it possible to arrive at the total and water-soluble volatile fatty acid content of the butters.

The data obtained on 69 samples of salted butter are outlined in table 1. In this table the data are averaged together on the basis of half-point divisions in score. This type of grouping has the disadvantage of uneven weighting, as the groups vary considerably in the number of butters. In order to remedy this condition, a smaller number of groups, based on approximately equal quantities of butter (except in the last division), is presented in table 2.

It may be noted in tables 1 and 2 that inverse general relationships of varying degrees exist between butter scores and the free fatty acid concentration of the butter and butter oil expressed as acid degree, volatile acidity, and Knaysi-Guthrie number. The most clear-cut and consistent relationship existed between total volatile fatty acid and flavor score of butter. The aver-

TABLE 1

Relationship between flavor score, acid degree, and free volatile fatty acidity of salted commercial butter

Butter score	No. of butters	Volatile acidity of butter		Acid degree	Knaysi-Guthrie no.	Volatile acidity of butter oil	
		Total	Water-soluble			Water-soluble	Total
		(ml. N/20 acid/20 g.)	*(ml. N/20 acid/20 g.)*			*(ml. N/20 acid/20 g.)*	*(ml. N/20 acid/20 g.)*
93.0	17	0.97	0.64	0.65	0.21	0.55	0.87
92.5	5	1.15	0.71	0.66	0.60	0.62	1.05
92.0	5	1.31	0.83	0.79	1.00	0.59	1.03
91.5	2	1.27	0.80	0.55	1.00	0.67	1.08
91.0	5	1.51	1.02	1.10	0.90	0.78	1.20
90.5	3	1.40	0.82	0.98	1.00	0.75	1.17
90.0	8	1.81	1.24	1.20	1.00	0.95	1.43
89.5	3	2.03	1.38	1.83	1.83	1.18	1.87
89.0	9	2.07	1.43	1.37	0.89	1.01	1.54
88.5	5	2.33	1.57	1.89	0.90	1.07	1.72
88.0	2	2.83	2.08	0.61	1.00	1.89	2.33
86.5	2	5.08	3.43	3.35	3.30	1.85	2.22
< 85.0	3	7.33	6.04	6.82	9.33	5.66	6.94

age volatile acid titer of 17 butters scoring 93.0 was 0.97 ml. N/20 acid/20 g. butter, and for 16 butters scoring 89 to 88 the average total volatile acid titer was 2.25 ml. N/20 acid/20 g. (table 2). Also, a fairly good inverse relationship was shown between the free water-soluble volatile fatty acid content and the flavor score of butter.

The data in tables 1 and 2 also reveal that the acid degree and flavor score of butter appear to be related to some extent, but the relationship was not as consistent as that shown by the volatile acids of the butters.

TABLE 2

Relationship between flavor score, acid degree, and free volatile fatty acidity of salted commercial butter, using relatively even groupings

Butter score	No. of butters	Volatile acidity of butter		Acid degree	Knaysi-Guthrie no.	Volatile acidity of butter oil	
		Total	Water-soluble			Water-soluble	Total
		(ml. N/20 acid/20 g.)	*(ml. N/20 acid/20 g.)*			*(ml. N/20 acid/20 g.)*	*(ml. N/20 acid/20 g.)*
93.0	17	0.97	0.64	0.65	0.21	0.55	0.87
92.5 to 91.0	17	1.32	0.85	0.82	0.85	0.66	1.09
90.5 to 89.5	14	1.77	1.18	1.29	1.18	0.96	1.47
89.0 to 88.0	16	2.25	1.56	1.44	0.91	1.14	1.70
< 86.5 to no score	5	6.43	5.00	5.43	6.92	4.14	5.05

In general, based only on averaged values, as the butter quality became extremely poor, the Knaysi-Guthrie number showed a high value, while for the butter of excellent quality the Knaysi-Guthrie number showed a low value. However, in the area between excellent and extremely poor, the Knaysi-Guthrie number was irregular and insensitive.

Analysis of the data obtained with the butter oil in comparison to those obtained on butter (tables 1 and 2) reveals that the free volatile fatty acid content of the oil exhibits a similar, but less consistent, trend in its relationship to flavor score of the butter. This indicates that the free volatile fatty acids of the butter serum, in conjunction with those of the fat, must be considered in any study of this nature if the results are to be correlated with observations on the butter.

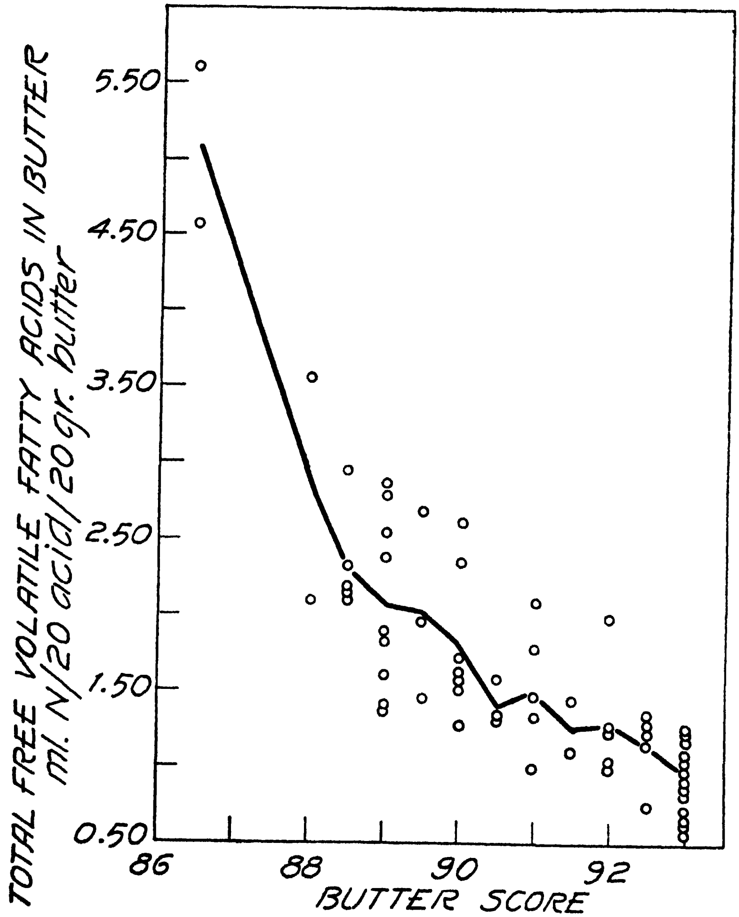

FIG. 1. The relationship between the total free volatile fatty acid content and the flavor score of salted commercial butter.

Although data shown in tables 1 and 2 appear on the whole to exhibit clear-cut relationships in some cases, it must be emphasized that these data represent averages and not individual values and, in most instances, there was a wide range of values within each flavor score division. This is to be expected in a study of this type where many factors cannot be controlled. Some butters may be scored down as a result of defects not associated with increased fatty acids. In the case of other butters, neutralized cream may produce an effect on acid degree determinations unrelated to concentration of free fatty acids. To show this point more clearly, part of the averaged data in table 1 was plotted on graphs, accompanied by results for the individual butters. Figures 1, 2, and 3 show the relationship existing, both in terms of averaged and individual values, between flavor score and total free volatile fatty acid content of butter; flavor score and acid degree; and flavor score and Knaysi-Guthrie number, respectively. Butter scores ranging from 93 to 86.5 were plotted on these graphs.

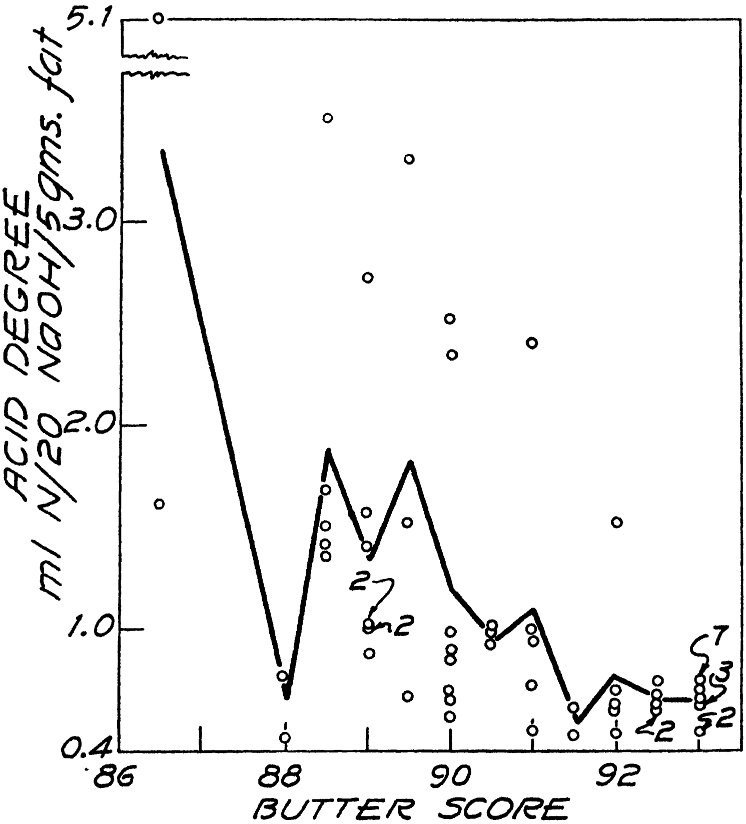

FIG. 2. The relationship between the acid degree and the flavor score of salted commercial butter. (The numbers in the figure show the times that the same data were obtained.)

It may be seen (fig. 1) that a definite inverse relationship does exist between the free volatile fatty acids and the flavor score of salted commercial butters. However, even this relationship, though definite in trend, is quite general when individual samples of butter are considered. The acid degree plotted against flavor score (fig. 2) shows wide variations between individual samples, although the general trend is for a good butter to have a low acid degree and a poor butter a high acid degree. The relationship of Knaysi-Guthrie number to flavor score of butter (fig. 3) generally was similar to that exhibited by the acid degree.

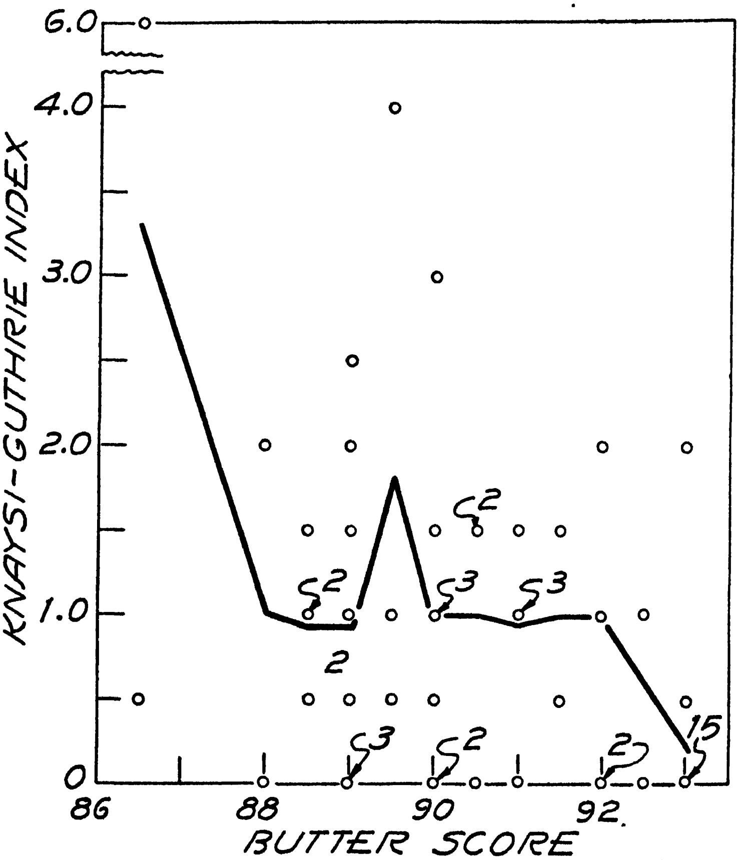

FIG. 3. The relationship between the Knaysi-Guthrie number and the flavor score of salted commercial butter. (The numbers in the figure show the times that the same data were obtained.)

DISCUSSION

A study was undertaken to clarify the situation in regard to the free fatty acid content of salted commercial butters and its relationship to flavor score.

Data obtained in this study tend to confirm, in part, the conclusion of Fouts (4, 6) in regard to acid degree, since only an inverse relationship of a very general nature was observed from an analysis of 69 salted commercial butters. This relationship may be disturbed by various factors. Fouts (5) found that when the titratable acidity of sour cream was reduced by the addition of an alkali, the acid number of the fat also was reduced but not proportionately. In the present investigation, several low-scoring samples of butter possessing extremely high concentrations of free volatile fatty acids showed surprisingly low acid degrees. Another factor which might upset this relationship would be the grading down of a butter because it had absorbed flavors from its environment or because it had other flavor defects not associated with fatty acids. Of 69 samples analyzed, only two of the butters were considered as having slight absorbed flavors. Naturally, the variation in the number of these types of butter would affect any relationship proportionately. However, this kind of butter probably represents only a very small percentage of the whole.

Studies on the free volatile fatty acids of butter show that these acids are allied with flavor deterioration to some extent. This appears logical, since it is well known that lower chain fatty acids, such as butyric, caproic, and caprylic, usually are associated with odors of a rancid nature in butter.

The new method evidently recovered more volatile fatty acids from butter than previous methods, and this was an important factor in showing the relationship between free volatile acids and butter score more clearly than heretofore. For example, Ferris *et al.* (3) found that the average free volatile acidity for 14 samples of 93–94 score butters was 0.65 N/10 acid per 100 g. of butter, whereas by the method used for this work, 17 samples of butter scoring 93 averaged 2.43 ml. N/10 acid per 100 g. of butter, an increase of more than three times. In this respect, Fouts (6), using a steam distillation method, found that for a series of butters exhibiting slight to pronounced rancid flavor, the percentage of total free fatty acid that was volatile ranged from 11.4 to 16.7 per cent. In contrast, from sweet-cream butters used in the current work, an average percentage recovery of approximately 30 per cent was obtained for all butter oils, again indicating more complete recovery. This value of 30 per cent was in good agreement with that obtained by Gould and Johnson (7) on fresh fat churned from milk and ether-extracted before steam distillation.

Neutralization of sour cream for buttermaking should not affect the free volatile acid value of the butter in the same way it affects the acid degree. This is due to the fact that in the process of recovery of the free volatile fatty acids, the salts of the fatty acids formed by a neutralizer would be split by acidification to pH 2. However, neutralization might be a factor in reducing some of the free volatile acids of butter as the result of increased possibilities of losses of the neutralized acid during the washing of the butter. The indication is that this effect could not be great.

In regard to the free volatile fatty acids in the serum, it was assumed that the butter existed in a ratio of four parts of butter oil to one part of serum. Actually this ratio is not always found, but for commercial butters it will remain quite constant. It is evident that for routine determination of total volatile acidity in butter, the analysis should be made on the fat and serum from a given weighed sample.

Although the free volatile fatty acids show an inverse relationship to flavor score of butter, this relationship does not appear exact enough to warrant using it as a basis for determining the flavor score of butter. As a basis for roughly determining the excellent, good, or poor qualities of a butter, it might be acceptable.

The Knaysi-Guthrie number did not correlate well with the butter score because of its apparent lack of sensitivity in regions of low fatty acid concentration. In the method advocated by Knaysi and Guthrie (11) for butter, the authors stress the fact that no perfect agreement should be expected between the quality of butter as estimated by their method and the score of the expert. The data presented herein show that 94 per cent of all butters gave Knaysi-Guthrie numbers of 2 or less and flavor scores ranging from 86 to 93, which substantiates the conclusion of Knaysi and Guthrie (11) who stated that "a test of 2 or below indicates, almost always, butter of fair, good, or excellent quality".

SUMMARY

The existence of a possible relationship between butter quality and free fatty acid concentration, including those of a volatile nature, was investigated. Methods involved included the Knaysi-Guthrie method on butterfat, a titration method for acid degree, and a modified ether-extraction direct-distillation technic for free volatile fatty acid concentration in butter and butterfat.

Free volatile fatty acid concentration of butter determined either as total or water-soluble acidity was found, in general, to be inversely related to the flavor score of 69 lots of salted commercial butter. Individual samples varied appreciably from the averages.

The relationship between the acid degree and the butter score was not well defined. An inverse relationship was discernible but individual samples varied greatly.

It was not possible to observe the existence of a close relationship between the flavor score of commercial butter and the Knaysi-Guthrie number of butterfat, even in average values, except that very high-scoring butters generally gave low values.

On the basis of the data obtained, it would not be advisable to recommend using either the free total acidity or the free volatile fatty acid values of butter as an index for determining flavor scores of salted commercial butters,

due chiefly to variation of individual samples from the averages. For a rough classification of commercial butters as excellent, good, fair, or poor, the use of free volatile acid values might have merit. However, the accumulation of much more data would be required before even this rough classification could be established.

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THE VALUE OF GROUND WHOLE GRAINS VERSUS BY-PRODUCTS IN CONCENTRATE MIXTURES FOR DAIRY COWS[1]

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The nature of concentrate mixtures fed to dairy cows varies widely, depending upon the availability of ingredients. In regions where farm-grown grains are raised abundantly, the concentrate mixture consists primarily of corn, oats, or barley, and a protein supplement. Other areas depend to a greater extent on various by-product feeds to supply a part of the concentrate portion of the dairy ration. Regardless of the source of ingredients, widely different mixtures give satisfactory results under practical conditions. Consequently, it was of interest to learn the relative value of quite different types of concentrate mixtures when fed under similar conditions. Two mixtures were chosen, one composed largely of by-product feeds, and another containing a high proportion of farm-grown grains.

Several investigations (1, 2, 3, 4, 5, 6, 7, 8) have been conducted comparing the nutritive value of simple and complex concentrate mixtures. In general, the results have indicated that simple mixtures are practically equal to more complex mixtures. However, little research has been conducted to compare by-product feeds and farm-grown grains when composing a major portion of the concentrate mixture. The following series of experiments was designed primarily to study the effect on milk production of concentrate mixtures composed of feeds of different sources.

EXPERIMENT A

This investigation was a double reversal feeding trial consisting of three 7-week periods. Two groups of six cows each were used. These groups were equalized on the basis of breed, age, weight, stage of lactation, and average production during a 2-week preliminary period. There were four Holsteins, one Brown Swiss, and one Ayrshire in each group. During the sixth week of the experiment, one Holstein cow died of an internal hemorrhage so her mate in the other group also was removed from the experiment.

Group I was fed the Cornell test mixture especially developed for feeding cows on official test and containing 58 per cent of by-product feeds. Group II received a ground whole grains mixture containing 76 per cent of farm-

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grown grains. At the end of each 7-week experimental period both groups of cows were reversed without a transitional feeding period.

The formulas of the concentrate mixtures are shown in table 1. The analyses shown in the table are for the mixtures fed in experiment C, which was conducted a year later than experiments A and B. Although the same formulas were used in all three feeding trials, the analyses showed the Cornell test mixture fed in experiments A and B contained 20.0 per cent of total protein and 5.8 per cent of fat and was calculated to contain 16.2 per cent of digestible protein and 77.8 per cent of total digestible nutrients. The ground whole grains mixture fed in the first two experiments contained 20.3 per cent of total protein and 5.5 per cent of fat and was calculated to contain 16.9 per cent of digestible protein and 76.9 per cent of total digestible nutrients.

TABLE 1

Formulas and analyses of the concentrate mixtures used

Ingredients	Cornell test mixture	Check 22% mixture	Ground whole grains mixture	By-products mixture
	(lbs.)	*(lbs.)*	*(lbs.)*	*(lbs.)*
Ground yellow corn	340	370	500	......
Ground oats	370	300	600	
Linseed meal	200	240	350	
Wheat bran	360	200		370
Distillers' corn grains, dried	300	300		300
Coconut oil meal	300	240		
Ground wheat			200	
Ground soybeans			220	
Corn gluten feed				400
Hominy feed				500
Soybean oil meal, 41% protein		220		300
Molasses	100	100	100	100
Dicalcium phosphate	15	15	15	15
Ground limestone	5	5	5	5
Salt	10	10	10	10
Total amount, lbs.	2,000	2,000	2,000	2,000
Total protein, %	18.47	22.15	17.77	22.90
Fat, %	5.27	5.18	5.44	5.58
Digestible protein, %	14.93	18.06	14.86	18.40
T.D.N., %	76.89	77.48	78.71	79.96

U. S. no. 2 clover-timothy mixed hay that analyzed 13.1 per cent of total protein and fairly well-eared corn silage supplied the roughage part of the ration. Hay was fed at the rate of 1 per cent and silage at the rate of 3 per cent of initial body weight. The concentrate mixtures were adjusted at weekly intervals in accordance with production the previous week. Concentrates were fed three times daily at the rate of 1 lb. per 3.5 lbs. of 4 per cent fat-corrected milk (F.C.M.). The experimental animals were milked three times a day.

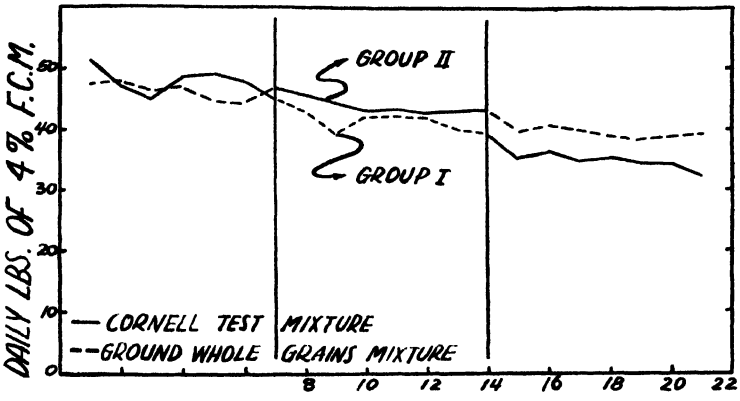

FIG. 1. The trend in milk production of the two experimental groups when receiving different concentrate mixtures.

Figure 1 shows the trend in milk production. There was very little difference between the two concentrate mixtures in promoting milk production. The average production of both groups on the Cornell test mixture shown in table 2 was 42.7 lbs. of 4 per cent F.C.M. and 42.3 lbs. of 4 per cent F.C.M. on the ground whole grains mixture. The difference in production of the groups on the two concentrate mixtures was not significant statistically. Since the difference was less than 0.5 lb. of milk per cow per day, it is likewise of little practical importance.

There was an average total gain in body weight for both groups of 3 lbs. per cow when the Cornell test mixture was fed, and an average total loss of 7 lbs. per cow during the periods when the ground whole grains mixture was fed. There was little difference in the consumption of concentrates, hay, and silage on the two rations.

From these results it appears that both concentrate mixtures were of nearly equal value when compared on the basis of milk production and maintenance of body weight. The difference in palatability, if any, was too small to be brought out by this experiment. Both mixtures were palatable enough

TABLE 2

Daily production of 4 per cent fat-corrected milk by experimental groups

	No. of cows	Cornell test mixture (control)	Ground whole grains mixture (experimental)
		(lbs.)	*(lbs.)*
Experiment A			
Double reversal trial (21 weeks)	10	42.7	42.3
Experiment B			
Continuous trial (26 weeks)	8	37.4	35.1

that the cows consumed all of the concentrates that were offered throughout the experiment.

EXPERIMENT B

A continuous feeding trial with two groups of six cows each was designed to study any accumulative effects of the two concentrate mixtures used in experiment A. This study was conducted simultaneously with the first experiment and extended over a period of 26 weeks. During the trial one cow in each group had to be dropped from the experiment, so the mate of each of these cows also was removed.

The experiment was summarized using only the four cows that completed the feeding trial in each group. The cows received the same general treatment and were fed at the same rates as those in experiment A. As this was a continuous trial, group III received the Cornell test mixture (table 1) for the entire 26 weeks, and group IV received the ground whole grains mixture for the same period. The trend in milk production is shown in figure 2.

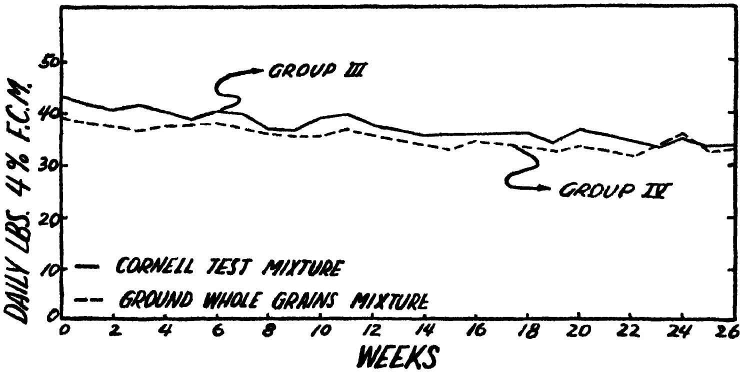

FIG. 2. The trend in milk production of the two experimental groups during a continuous feeding trial of 26 weeks.

During the experimental period, group III produced an average of 37.4 lbs. of 4 per cent F.C.M., while group IV averaged 35.1 lbs. of 4 per cent F.C.M. per day (table 2). This difference of 2.3 lbs. in favor of the group receiving the Cornell test mixture does not indicate necessarily any superiority of that mixture in feeding value. One more accurately might assign this difference in production to the fact that group III, which received the Cornell test mixture, was a higher-producing group. During the 2-week preliminary period, this group averaged 2.8 lbs. more milk than did group IV. Figure 2 shows that the trend in milk production was essentially the same. The difference existing between the groups at the end of the feeding trial was slightly less than at the beginning of the experiment. The body weight changes were of greater magnitude but were in the reverse order of those that occurred in experiment A. The cows in group III lost an average of 22 lbs. during the 26 weeks while receiving the Cornell test mixture, and group IV showed an average gain in body weight of 12 lbs. per cow.

EXPERIMENT C

Four groups of six cows each were selected for this reversal type of experiment. Three Holsteins, one Brown Swiss, and two Jerseys were in each group. Four concentrate mixtures were fed in rotation to each group of cows in order to eliminate the effect of differences in production among the groups. The allotment of the cows to the various groups was based on breed, age, weight, stage of lactation, and expected productive ability.

The formulas and analyses of the concentrate mixtures fed are given in table 1. The Cornell test mixture and the ground whole grains mixture had been used earlier in experiments A and B. Two additional mixtures which contained approximately 22 per cent of total protein also were included. The by-products mixture consisted entirely of by-product feeds and minerals and contained 22.9 per cent of total protein. The check 22 per cent mixture was fed as a control mixture to determine the effect, if any, of a slightly higher protein mixture, since the two mixtures first mentioned contained about 18 per cent of total protein.

These two latter mixtures contained a larger amount of protein for two practical reasons. First, mixtures of such protein content fit the type of roughage fed on many farms, especially where non-leguminous roughages are fed. Second, it is difficult to make up a satisfactory mixture of by-products alone that has less than 20 per cent of total protein.

The average grade of the hay fed was no. 2 timothy medium clover mixed hay, and this hay contained 10.2 per cent of total protein. The corn silage was of excellent quality and was fairly well-eared.

The experimental periods were 6 weeks in length. At the end of each period the concentrate mixtures were changed abruptly with no intervening transition. The cows were milked three times daily, and the concentrates were fed previous to each milking. Hay and silage were fed twice daily. Concentrates were fed at the rate of 1 lb. for each 3.5 lbs. of 4 per cent F.C.M. produced daily during the previous week. One pound of hay was fed for each 100 lbs. of body weight. Slightly more than 3 lbs. of corn silage per day per 100 lbs. of body weight were fed. Daily weighbacks of uneaten hay were recorded, but it was unnecessary to take weighbacks of concentrates or silage.

It was somewhat surprising that the concentrate mixtures that differed so much in their ingredients were eaten so readily when the cows were changed abruptly from one mixture to another. Judging from the rate at which the cows consumed the mixtures, it must be assumed that they were unable to detect or were indifferent to the overnight changes that occurred at the end of each experimental period. There were no observable differences in the palatability of the four concentrate mixtures.

Table 3 gives a summary of the production of 4 per cent F.C.M. This table summarizes the production on the basis of concentrate mixtures and groups of cows. The highest average production, 42.9 lbs. of 4 per cent F.C.M. per cow per day, was obtained when the groups received the check 22 per cent mixture. The average production was 42.5 lbs. when the Cornell test mixture was fed; 42.4 lbs. of 4 per cent F.C.M. were produced when the ground whole grains mixture and the by-products mixture were fed. The greatest average difference in production on these four mixtures was only 0.5 lb. of milk per day. This difference was not statistically significant. The variation in production on the different concentrate mixtures was considerably less than the differences among the experimental groups. Also, there was less difference in production on the Cornell test mixture and the ground whole grains mixture than was shown in the double reversal trial in experiment A.

TABLE 3

Summary of the daily production of 4 per cent fat-corrected milk

Group	Av. production of 4 per cent F.C.M. per day				
	Cornell test mixture	Check 22% mixture	Ground whole grains mixture	By-products mixture	Av. production
	(lbs.)	*(lbs.)*	*(lbs.)*	*(lbs.)*	*(lbs.)*
I	47.2 (period 1)	43.6 (period 2)	40.5 (period 3)	36.0 (period 4)	41.8
II	35.2 (period 4)	46.1 (period 1)	43.7 (period 2)	39.2 (period 3)	41.1
III	43.2 (period 3)	40.1 (period 4)	47.3 (period 1)	46.5 (period 2)	44.3
IV	44.2 (period 2)	41.7 (period 3)	38.1 (period 4)	47.8 (period 1)	43.0
Av. production	42.5	42.9	42.4	42.4	42.6

Feed consumption was practically the same for all groups. Since the concentrate portion of the ration was fed according to production, the higher producing groups received slightly more concentrates. The change in body weight was a general trend toward a slight gain in body weight throughout the experimental period.

DISCUSSION

This series of experiments has several practical implications. In periods when some of the standard ingredients in feed mixtures are difficult to obtain, the results of these experiments indicate that substitutions may be made on a rather wide basis without altering the feeding value or palatability so long as the total protein and total digestible nutrients remain fairly constant. If it is economical to do so, the results indicate that by-product feeds of the vegetable oil-producing and milling industries may replace farm-grown grains entirely, and vice versa, to a certain extent, without noticeably affecting the nutritive value of the mixture. In fact, such a procedure is rather common practice among feed mixers in producing economical feeds from ingredients that change in their price relationships to one another from time to time. Although not a commonly recommended feeding practice, abrupt changes in the concentrate mixture may be effected without producing harmful results.

SUMMARY

A series of three experiments comparing the feeding value of different concentrate mixtures indicated that there was little or no difference in the palatability of concentrate mixtures that differed widely in the ingredients used. A mixture containing 76 per cent of farm-grown grains was equal in promoting milk production and in palatability to a standard concentrate mixture throughout a continuous study of 26 weeks' duration. Similar results were obtained in a double reversal trial.

Another experiment involving four concentrate mixtures containing widely different ingredients showed there was little difference in feeding value among the four mixtures. Abrupt changes from one mixture to another had no unfavorable effect on feed consumption. All mixtures proved equally palatable. Body weight essentially was unaffected by the different concentrate mixtures.

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THE HEAT RESISTANCE OF LACTOBACILLI FOUND IN AMERICAN CHEDDAR CHEESE[1]

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The heat treatment of milk for cheesemaking is not a new development. However, pasteurization as ordinarily defined has not been used extensively in the cheese industry, the temperature and time of exposure often being less than the minimum required by public health officials. A number of states have passed laws requiring that cheese either be made from pasteurized milk as defined by public health ordinances or be aged a specified length of time. Such laws affect not only the cheese made in these states but also all the cheese produced in other states for sale in the areas concerned.

Experience has shown that Cheddar cheese made from pasteurized milk rarely develops the full, characteristic flavor ordinarily found in good quality raw milk cheese, even after an extended ripening period (11, 19, 20). This may be due to the destruction of enzymes, destruction of microorganisms, or to a chemical change in the milk as a result of pasteurization. Bacteriological studies have shown that lactobacilli often grow extensively in Cheddar cheese and frequently are present in tremendous numbers after ripening for several weeks (1, 15, 16, 17, 21, 22, 23). This suggests that these organisms may be important in the cheese-ripening process. The reason pasteurized milk cheese ripens more slowly may be that many of these organisms are destroyed in the pasteurizing process. The object of this investigation was to study the heat resistance of lactobacilli found in Cheddar cheese.

REVIEW OF LITERATURE

The standard of comparison of heat tolerance of different species of bacteria originally was the thermal death point, *i.e.*, the lowest temperature at which a suspension of bacteria could be killed in 10 minutes. This method cannot give comparable results unless conditions such as age of culture, approximate number of cells, pH of suspension, dimensions of test tubes, and thickness of glass in the test tubes are standardized.

The idea has since gained acceptance that (a) there is no critical lethal temperature, (b) any temperature high enough to have an unfavorable effect upon the growth and stamina of the bacteria is lethal and (c) bac-

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[1] Taken from data presented in a thesis to the Graduate Faculty of the University of Minnesota by W. L. Slatter in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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teria will be destroyed if they are subjected to an unfavorable temperature long enough. Research workers in the canning industry have found it more suitable for their purposes to keep the temperature constant and to vary the time. The thermal death time is considered to be the shortest time necessary to kill all bacteria in a given suspension at a given temperature.

Since the basic work of Kröng and Paul (19) in 1897, it has been recognized that the mortality rate of bacteria exposed to unfavorable conditions follows a generally regular and consistent course. Under a wide variety of conditions, this course is such that a straight line is obtained when the log of the number of bacteria surviving at a given moment is plotted against the time elapsed since the beginning of the experiment.

The order of death of spore-forming bacteria has been found to be logarithmic by Chick (10), Bigelow (6), Weiss (27), Esty and Meyer (11), and Watkins and Winslow (26).

Bigelow and Esty (8) were perhaps the first to consider some of the factors now recognized as very important, and they proposed a standard technic for determining the resistance of organisms to heat. They proposed an accurately controlled oil bath and special thermal-death-time tubes of soft glass 250 mm. long, 7 mm. inside diameter, and with a wall thickness of 1 mm. The spores were suspended in juices expressed from various canned foods which had received one heat treatment. When a juice had been inoculated with a spore suspension, it was introduced into the thermal-death-time tube, which then was sealed and placed in an oil bath for the heat treatment. Fifteen seconds were allowed for the tubes to come to bath temperature before time was counted. This method was designated as the "single tube" method in contrast to one proposed later by Esty and Williams (12). One difficulty with the single tube method was described as "skips", *i.e.*, destruction of organisms at shorter times when longer heating would leave viable cultures.

In 1924 Esty and Williams (12) introduced a "multiple tube" method to reduce the number of skips. Instead of heating one tube for a given time, 25 to 30 tubes were heated, each containing a portion of the same suspension, and all were heated alike for at least four different time intervals. These intervals were selected to cover the entire range of heat resistance based on percentage survival.

Bigelow (6), using the thermal-death-time data reported by Bigelow and Esty (8), plotted the results on semi-logarithmic paper instead of coordinate paper. The curves were drawn so that they passed between the time intervals representing the last positive and the first negative thermal death tubes in the greatest number of pairs of observation points with each organism. Such curves were straight lines or nearly so, and from them was secured the thermal death time of the organism between the temperatures which were used to construct the curve. Bigelow's work was done with spore-forming, thermophilic organisms. Since no data were available for non-spore-forming bacteria, he determined the heat resistance of four such organisms (*Bacterium alkaligenes*, *Bacterium coli*, *Bacterium aerogenes*, and *Bacterium proteus*) at temperatures of 40° C. (104° F.) to 65° C. (149° F.) at 5°-intervals. He did not find as consistent results with these organisms as he did with the spore-forming, thermophilic bacteria. However, he concluded that the thermal-death-time curves for these organisms were logarithmic.

Since Bigelow's suggestion that death of non-spore-forming bacteria was logarithmic, Watkins and Winslow (26), Beamer and Tanner (5), and Baker and McClung (2) have published confirmatory results.

Since bacteria follow a more or less uniform logarithmic order of death, death rates can be computed and conclusions drawn from them. Bigelow, Bohart, Richardson, and Ball (7) described the "general method" for making calculations of processing times for canned foods. One of the requirements was that the thermal death time of the organisms being destroyed by the process must be known at all temperatures attained during the process. This knowledge was obtained by determining the thermal death times at several temperatures in the processing range. The data so obtained were plotted, using a logarithmic time scale and a linear temperature scale. The resulting points were connected by a smooth curve. From this curve, thermal death times were found for all temperatures obtained during the heating process. It was not necessary to know the mathematical formula relating the thermal death time to the temperature.

Ball (3, 4) suggested some improvements for the "general method" of process calculations used in the study of temperatures required in the canning of vegetables. He did not use death rates or temperature coefficients but the factors F and z. The value F_1 was the thermal death time of the bacterial species at 121° C. (250° F.), while the value F represented the thermal death time at any other temperature. The letter z referred to the temperature increase in degrees Fahrenheit necessary to reduce the death time one-tenth. The value of z indicated the slope of the straight line obtained by plotting the logarithms of death time against temperature. The value F gave one point on the curve. Therefore, F and z were sufficient to characterize the thermal resistance of the bacterial spores at any temperature.

When making thermal-death-time tests involving relatively short times (less than 10 minutes), the heat penetration lag, or the time for the thermal-death-time tube and its contents to come up to the temperature of the constant-temperature bath, may make up an appreciable percentage of the total death time (24). Many investigators apparently have ignored this fact. Bigelow and Esty (8), Weiss (27), Esty and Meyer (11), and Esty and Williams (12) used a series of glass tubes containing suspensions of heat-resistant bacteria which were heated in oil baths. Not more than 15 seconds were allowed for lag in heat penetration. Townshend (25) measured the heat resistance of spore-forming anaerobes, using a water bath. He allowed a lag correction of 1 minute in the heating times. Sognefest and Benjamin (24) measured the heating lag in Pyrex thermal-death-time tubes by means of a thermocouple when various media such as water, sugar solutions, and vegetable juices were placed in the tubes. The correction factor for water figured for an organism with a *z* value of 18 was 0.85 minute when heated in a water bath. Gross (14) used Kimble brand no. 45050 chemical test tubes in thermal-death-time studies of a staphylococcus in a meat-juice medium heated in an oil bath. The lag on these tubes was measured by means of a thermocouple and found to be approximately 3 minutes when heated to 140–160° F.

EXPERIMENTAL METHODS

Samples of cheese were obtained, using sterile triers, and the samples transferred to sterile sample jars. An 11-g. sample of each cheese was transferred to a sterile mortar and ground to a homogeneous suspension with the aid of a pestle, a small amount of sterile sand, and the addition of part of the water from a 99-ml. sterile water dilution blank, the mixture representing a 1 to 10 dilution of the cheese. From this dilution, other desired dilutions were prepared. The various dilutions then were plated, using Difco tomato juice agar containing 400 ml. tomato juice, 10 g. Bacto peptone, 10 g. Bacto peptonized milk, and 11 g. Bacto agar per liter. The plates were incubated at room temperature (21° C.) for 10 days. Twenty-five contiguous colonies were picked from one plate in each set and inoculated into tubes of sterile litmus milk. After 10 to 14 days incubation at room temperature, the appearance of each litmus milk culture and the morphological characteristics of the organism were recorded. The cultures were stained with the gram stain using the Burke (9) modification. Non-spore-forming, gram-positive rods that reduced and coagulated litmus milk in 10 to 14 days at room temperature were selected as lactobacilli. The length and width of cells and the speed of growth in litmus milk were considered in the selection of cultures for the heat-resistance studies. Not more than two cultures were selected from any one cheese. The cultures selected were streaked on tomato juice agar, covered with another layer of agar, and incubated for 10 days at room temperature. Colonies then were picked into litmus milk and incubated again for 10 days at room temperature. The morphology of these cultures was observed, using the gram stain, and one culture was selected for the heat-resistance studies. The cultures selected were inoculated into glucose, galactose, lactose, fructose, maltose, mannite, mannose, salicin and inulin broths.

The cultures were numbered as follows: the first number refers to the number of the cheese in which the organism was found, the second to the number of the colony picked from the plate used in the isolation, and the third to the colony picked when the cultures were streaked on tomato juice agar. A record was kept of morphology, colony, and fermentation characteristics so that it was possible to get the history of a culture whenever necessary.

The method employed for the heat-resistance studies was similar to that used by Bigelow and Esty (8), with litmus milk made from fresh skim milk. One drop (from a 2.2-ml. pipette) of each lactobacillus culture was transferred into a 10-ml. sterile, skim milk dilution blank. The dilution blank was shaken vigorously for 1 minute. One milliliter of this dilution was added to each 100 ml. of sterile litmus milk to be inoculated for the heat treatment. The inoculated litmus milk was allowed to remain over night at 5° C. and then transferred to sterile, Kimble brand no. 45050 (10 × 75 mm.) chemical test tubes and the tubes sealed. The number of bacteria in the inoculum was determined by plating various dilutions of the litmus milk on tomato juice agar.

The sealed tubes were submerged in a De Khotinsky constant-temperature water bath with a maximum temperature variation of ± 0.2° F. The tubes were exposed in the constant-temperature bath for varying periods of time, the time intervals being measured with a stop watch. When the tubes were taken from the water bath, they were immersed at once in water at 60–65° F. to cool. The tubes then were allowed to incubate 4 weeks at 30° C., after which all tubes were observed for growth. The first negative tubes in a series frequently were plated or observed under a microscope to be sure no viable organisms were present. Single tubes were heated at 5-minute intervals to get a general picture of the heat resistance of the lactobacillus cultures. To determine the z values, ten tubes were removed at from 1- to 5-minute intervals at each of four different temperatures.

In order to determine the heat lag on the Kimble tubes used in the heat-resistance studies, a skim milk thermometer was made by attaching a 29-inch capillary tube to one of the Kimble tubes. A vacuum was pulled on the test tube with the capillary tube attached, and the test tube filled with skim milk containing a few drops of formaldehyde. It then was attached to a meter stick and calibrated against a mercury thermometer by holding in a water bath at different temperatures. To get the heat lag, this milk thermometer was placed in a constant-temperature bath at various temperatures and the meter stick reading recorded every 15 seconds. To get the cooling lag, the milk thermometer was transferred directly from the constant-temperature bath to water at 65° F. and the readings recorded every 15 seconds until it reached 65° F. The time of heating and cooling was converted to an equivalent time at the temperature of the constant-temperature bath by a method of graphic integration.

The temperature lag correction, when heating a skim milk medium in water to 135°, 145°, and 155° F., using z values from 8 to 12, was found

TABLE 1

Thermal death times at 145° F. of cultures from raw milk cheese

Period of exposure in minutes									
< 5	10	15	20	25	30	35	50	60	Over 60
Culture nos.									
3-8-5 4-17-4 7-13-6 9-7-2 9-17-1 12-9-2 18-1-5 18-19-6 21-11-6 22-7-4 23-19-1 2-1-7	11-9-5 16-14-1 24-14-4 24-20-5	6-15-5 11-14-5 15-27-1 16-11-3 17-21-1 21-8-1 25-4-6 32-1-1 32-6-1	7-14-6 23-1-3 25-15-6	10-1-8 10-2-8 12-27-6 13-26-6 14-5-1 20-6-6 22-19-6 2-14-6	15-5-1 3-13-4 4-27-6 6-25-7	8-6-3	8-16-3 20-8-1	17-6-3 19-1-6	13-20-6 14-4-4 19-27-6

to be approximately 1 minute. One minute was subtracted from the time that the tubes were exposed to the constant temperature to get the lethal time of exposure in the constant-temperature bath.

Thirty-two cheeses made in various parts of Wisconsin and Minnesota were the source of the organisms used in these experiments. Twenty-four of the cheeses were made from raw milk. Two cheeses were made from milk pasteurized at 145° F. for 30 minutes, while six were made from milk flash-heated, the temperature fluctuating from 155° to 170° F. The age of the cheese varied from 1 week to 2 years.

RESULTS

Eight hundred colonies were picked from the 32 cheeses; 448 colonies were of organisms of the rod type. Of the 25 colonies picked from a plate from each cheese, the types varied from all rods to all cocci. Very few rod types were found in the 2-year-old cheese. In the other cheese, many had a very high proportion of rods. When inoculated into litmus milk, 439 cultures were found to be acid coagulating, 345 acid non-coagulating, 12 formed a yellow sediment at the bottom of the litmus milk tubes, and 6 cultures digested the milk solids. Sixty cultures were selected for further study on the basis of morphology, growth in litmus milk, and the source of the culture (raw or pasteurized milk cheese).

A summary of the thermal death times at 145° F. of lactobacilli from raw milk cheese is given in table 1. Twelve cultures could be killed in less

TABLE 2

Thermal death times at 136° F. of cultures from raw milk cheese killed in less than 5 minutes at 145° F.

Period of exposure at 136° F.	Thermal death time of culture no.										
	18–1–5	12–9–2	9–17–1	9–7–2	21–11–6	18–19–6	23–19–1	22–7–4	3–8–5	4–17–4	7–13–6
Minutes											
0	+	–	+		+	+	+	+	+	+	+
5	+	–	+	+	+	+	+	+	+	+	+
10	+	–			–	+	–	+	–	+	+
15	+	–	+	+	–	+	–	–	–	–	–
20	+	–	+	–	–	–	–	–	–	–	–
25	+	–	–	–	–	–	–	–	–	–	–
30	+	–	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–	–	–
40	–	–	–	–	–	–	–	–	–	–	–
45	–	–	–	–	–	–	–	–	–	–	–
Plate count per ml. of litmus milk culture (thousands)	1	< 1	11	72	130	< 1	160	6	4	< 1	3

+ Indicates growth.
– Indicates no growth.

TABLE 3

Thermal death times at 154° F. of cultures from raw milk cheese not killed in 60 minutes at 145° F.

Period of exposure at 154° F.	Thermal death time of culture no.		
	13–20–6	14–4–4	19–27–6
Minutes			
0	+	+	+
5	+	+	+
10	−	−	−
15	−	−	−
20	−	−	−
Plate count per ml. of litmus milk culture	124,000	58,000	120,000

+ Indicates growth.
− Indicates no growth.

than 5 minutes, four in 10 minutes, nine in 15 minutes, three in 20 minutes, eight in 25 minutes, four in 30 minutes, one in 35 minutes, two in 50 minutes, and two in 60 minutes, while three cultures could not be killed in 60 minutes. Approximately 83 per cent of the cultures could be killed in 30 minutes or less at 145° F.

The thermal death times at 136° F. of cultures from raw milk cheese, which could be killed in less than 5 minutes at 145° F., are given in table 2. The thermal death time varied from less than 5 minutes to 35 minutes, with nine of the eleven cultures being killed in from 10 to 20 minutes.

The thermal death times at 154° F. of cultures from raw milk cheese not killed in 60 minutes at 145° F. are given in table 3. All three of these cultures could be killed in 10 minutes at 154° F.

TABLE 4

Thermal death times at 154° F. of cultures from pasteurized milk cheese

Period of exposure at 154° F.	Thermal death time of culture no.									
	5–6–6	5–8–9	26–2–1	26–6–1	27–2–1	27–19–1	28–2–1	28–16–2	30–1–1	30–20–1
Minutes										
0	+	+	+	+	+	−	+	+	+	+
5	+	+	+	+	+	−	+	+	+	+
10	+	+	+	+	+	−	−	+	+	+
15	+	+	+	−	−	−	−	+	+	+
20	+	+	+	−	−	−	−	−	−	+
25	+	−	−	−	−	−	−	−	−	−
30	−	−	−	−	−	−	−	−	−	−
35	−	−	−	−	−	−	−	−	−	−
40	−	−	−	−	−	−	−	−	−	−
Plate count per ml. of litmus milk culture (thousands)	100	130	235	17,000	100	3.800	51	122	73	3,000

+ Indicates growth.
− Indicates no growth.

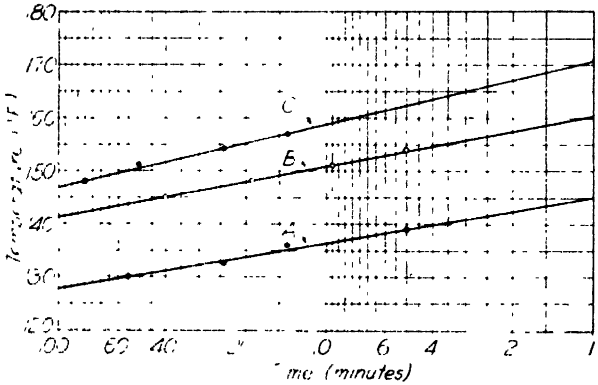

FIG. 1. Thermal-death-time curves: Curve A, Culture no. 3-8-1, *z* value = 8.5. Curve B, Culture no. 8-16-3, *z* value = 9.5. Curve C, Culture no. 5-6-6, *z* value = 12.

The thermal death times at 154° F. of cultures from pasteurized milk cheese are given in table 4. The thermal death times varied from 10 to 30 minutes. The colonies of this group of organisms on tomato juice agar commonly were very small, approaching pin-point size.

The fermentation characteristics of all cultures isolated were studied and found to have little or no correlation with their heat resistance.

The heat resistance of the organisms was the only factor considered in selecting the cultures for the thermal-death-time curve studies. Two cultures (3-8-1 and 11-9-5) having a low heat resistance, three (8-16-3, 6-25-7, and 19-1-6) having a medium heat resistance, and two (5-6-6 and 30-20-1) having the maximum heat resistance were selected.

Culture no. 3-8-1 could be killed within 5 minutes at 139°, 14 minutes at 136°, 24 minutes at 133°, and 55 minutes at 130° F. When these data

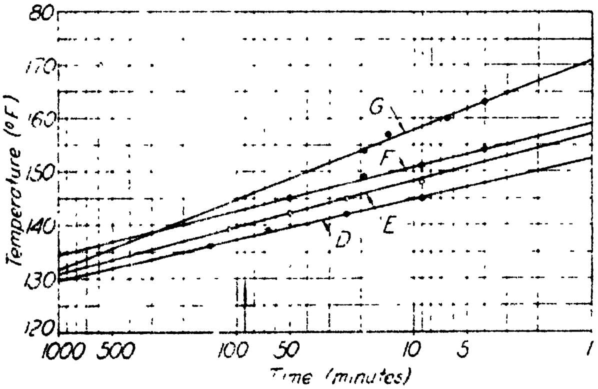

FIG. 2. Thermal-death-time curves: Curve D, Culture no. 11-9-5, *z* value = 8. Curve E, Culture no. 6-25-7, *z* value = 8. Curve F, Culture no. 19-1-6, *z* value = 8.5. Curve G, Culture no. 30-20-1, *z* value = 13.

were plotted on semi-logarithmic paper, they gave curve A shown in figure 1. The *z* value (slope of the curve) for this culture was approximately 8.5.

Culture no. 8–16–3 could be killed in 5 minutes at 154°, 9.5 minutes at 151°, 19 minutes at 148°, and 40 minutes at 145° F. When these data were plotted, they gave curve B (fig. 1). The *z* value was approximately 9.5.

Culture no. 5–6–6 could be killed in 14 minutes at 157°, in 24 minutes at 154°, in 50 minutes at 151°, and in 80 minutes at 148° F. These data gave thermal-death-time curve C (fig. 1). The *z* value was 12.

Culture no. 11–9–5 could be killed within 9 minutes at 145°, 24 minutes at 142°, 65 minutes at 139°, and 140 minutes at 136° F. These data gave curve D (fig. 2). The *z* value was 8.

Culture no. 6–25–7 could be killed within 9 minutes at 148°, in 24 minutes at 145°, in 50 minutes at 142°, and 110 minutes at 139° F. These data gave curve E (fig. 2), indicating this culture had a *z* value of approximately 8.

Culture no. 19–1–6 could be killed in 4 minutes at 154°, 9 minutes at 151°, 19 minutes at 149°, and 50 minutes at 145° F. These data gave curve F (fig. 2). The *z* value was 8.5.

Culture no. 30–20–1 could be killed in 4 minutes at 163°, 6.5 minutes at 160°, 14 minutes at 157°, and 19 minutes at 154° F. These data gave curve G (fig. 2), indicating a *z* value of approximately 13.

The lactobacilli that could be killed in 30 minutes or less at 145° F. had *z* values varying from 8 to 8.5, while the organisms that could be killed in from 30 to 60 minutes at 145° F. had *z* values from 8.5 to 9.5. The organisms having a thermal death time of over 60 minutes at 145° F. had *z* values varying from 12 to 13.

DISCUSSION

The heat resistance of lactobacilli found in Cheddar cheese varied within rather wide limits. Some of this variation undoubtedly was due to the differences in the numbers of organisms in the cultures used for the heat-resistance trials. However, this variation was not great when the thermal death time was less than 30 minutes at any temperature. It usually was possible to take different cultures of the same strain of lactobacilli and have the thermal death time on successive trial runs check within 5 minutes. When the thermal death time was over 30 minutes, a much greater variation frequently was observed. Since the number of lactobacilli in a normal raw milk supply is comparatively low (1), the heat resistance data reported in this study may suggest a greater heat resistance for some organisms than would actually be the case in a raw milk supply. This observation seems justified because of the large numbers of organisms present in some of the cultures used for these heat-resistance trials.

In this study the percentage of lactobacilli destroyed by pasteurization exposure was considered more important than the variability of the heat resistance. Approximately 83 per cent of the lactobacilli found in Cheddar cheese made from raw milk could be killed in 30 minutes or less at 145° F. The lactobacilli found in Cheddar cheese made from pasteurized milk were much more heat resistant, having a thermal death time of from 10 to 35 minutes at 154° F. This might explain why Evans, Hastings, and Hart (13) found only one-tenth as many *Lactobacillus casei* in pasteurized milk cheese as in raw milk cheese made from the same milk up to the forty-second day of ripening. The destruction of lactobacilli by pasteurization may be an important reason why cheese made from pasteurized milk ripens more slowly than cheese made from raw milk. This suggests the possibility that the ripening of cheese made from pasteurized milk may be accelerated either by adding the proper lactobacillus cultures or by ripening the cheese at a higher temperature in order to supply a more favorable growth temperature for the reduced numbers of lactobacilli that survive pasteurization.

The fermentation characteristics of the lactobacilli found in Cheddar cheese had very little correlation with heat resistance. When the organisms were grouped according to their heat resistance, the more organisms in any one group the fewer the fermentation characteristics they had in common.

The temperature lag on the Kimble brand test tubes used was approximately 1 minute in a water medium. This checks closely with the correction determined by Gross (14) using the same tubes and by Sognefest and Benjamin (24) using similar tubes.

The *z* values for the lactobacilli varied from eight to thirteen, with the most heat-resistant organisms having the highest *z* values. A thermal-death-time curve with a slope of 8 passing through a point at 145° F. for 30 minutes shows that the lactobacilli which could be killed in 30 minutes or less at 145° F. also could be killed in 27 seconds at 160° F. or 7 seconds at 165° F. This would include 83 per cent of the lactobacilli found in Cheddar cheese made from raw milk, as milk for cheesemaking commonly is pasteurized at 165° F. for 15 seconds. Most ordinances require a minimum time and temperature exposure of 143° F. for 30 minutes or 160° F. for 15 seconds for public health reasons. These heat exposures would destroy approximately 52 per cent of the bactobacilli found in raw milk cheese. Using the minimum exposures would permit a significant increase in the number of lactobacilli surviving pasteurization.

Some skips were encountered, especially where the thermal death time was over 1 hour. Few skips were encountered when the thermal death time was less than 30 minutes at any temperature.

SUMMARY AND CONCLUSIONS

1. The heat resistance of 60 lactobacillus cultures found in Cheddar

cheese made from raw milk was studied and found to vary within wide limits.

2. The majority of lactobacilli found in Cheddar cheese made from raw milk can be killed by pasteurizing at 143° F. for 30 minutes or 160° F. for 15 seconds.

3. The fermentation characteristics of the lactobacilli found in Cheddar cheese had little correlation with their heat resistance.

4. The *z* values vary in a general way with the heat resistance of the organisms, the most heat-resistant lactobacilli having the highest *z* values.

5. The destruction of lactobacilli by pasteurization suggests the possibility of accelerating the ripening of Cheddar cheese made from pasteurized milk either by adding the proper lactobacillus culture to the milk or by raising the ripening temperature of the cheese to supply a more favorable growing temperature for the reduced numbers of lactobacilli that survive pasteurization.

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THE STABILIZATION OF CAROTENE IN DEHYDRATED LEGUMES (ALFALFA) AND CEREAL GRASSES[1, 2]

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In an earlier study on the stabilization of carotene (2) in dehydrated feeds and foods, consideration of various physical and chemical treatments was made.

Additional heat treatments were effective in reducing the loss of carotene in dehydrated oats from 70–80 per cent to 30–50 per cent in 6 months storage. The addition of 0.9 per cent of diphenylamine to dehydrated oats decreased the loss of carotene from 77 to 41 per cent in 6 months. Experiments with a number of chemical agents which changed the reaction of the material or of substances which could act as reducing agents or acceptors of oxygen were ineffective. Pelleting and coating with flexo wax reduced the loss from 74 to 45 per cent in 6 months. Attempts to remove the oxygen from the pellets before coating by washing with nitrogen also helped materially in decreasing the carotene loss.

Further studies (3) showed that autoclaving dehydrated oats or alfalfa at 15 lbs. pressure for 1 hour and then pressing into large pellets (3 × 4 inches) and dipping in flexo wax, reduced the loss in dehydrated alfalfa to 28 per cent in 3 months and to 0 per cent in the case of dehydrated oats.

EXPERIMENTAL

Additional studies now have been made on the influence of time of autoclaving on the stabilization of carotene, as well as pelleting and nitrogen washing before pelleting and waxing. In table 1 the data show the effect of autoclaving for 1 hour in the absence of oxygen. In this process the chlorophyll is destroyed and the material is of dark brownish color. Carotene determinations were made by the method outlined previously (1). These data show that mere autoclaving to assure complete destruction of the "lipoxidase", which can bring about the destruction of carotene, had no significant effect in checking carotene losses. Only when oxygen is excluded, either in part or, preferably, completely, is preservation of the carotene brought to a comparatively high percentage. Washing with nitrogen before pressing was quite effective in further reducing the loss. However, after washing in nitrogen, the material was exposed to air in the process of pellet making.

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In table 2 are the records of the effect of time of autoclaving on the carotene conservation. These data show that autoclaving for 5 minutes gave results similar to those secured with 60 minutes of autoclaving; carotene was well preserved in the material that had not been autoclaved at all but protected from oxygen through pelleting and waxing, or, even better, by washing with nitrogen and then pelleting in air and waxing. These data are in harmony with earlier observations by other investigators. The carotene can be preserved in plant tissues where the dehydrated material is stored in an inert gas such as nitrogen (1). Mere destruction of the carotene oxidizing enzyme, lipoxidase, will not lead to carotene preservation. Oxygen must be excluded or auto-oxidation of the carotene will proceed.

TABLE 1

Effect of autoclaving for 1 hour on the stability of carotene in dehydrated cereal grasses and alfalfa kept at 22–25° C.

(All samples pressed[1] into large pellets 3 × 4 in.)

Material and treatment	Carotene content, µg./g. (air-dry basis)			% loss 6 mos.
	Initial	3 mos.	6 mos.	
Dehydrated oats				
No treatment	450	155	116	74.2
Autoclaved	423	171	74	82.5
Auto. + flexo waxed	423	379	335	20.8
Auto. + flexo waxed + N_2 washing	423	417	368	13.0
Dehydrated sudan grass				
No treatment	299	96	83	72.2
Autoclaved	323	175	136	57.9
Auto. + flexo waxed	323	191	171	47.0
Auto. + flexo waxed + N_2 washing	323	260	256	20.7
Dehydrated alfalfa				
No treatment	222	88	72	67.6
Autoclaved	235	117	75	68.1
Auto. + flexo waxed	235	154	151	35.8
Auto. + flexo waxed + N_2 washing	235	160	173	26.5
Alfalfa dried at 50° C.				
No treatment	236	154	153	35.2
Autoclaved before drying	334	190	129	61.4
Auto. + flexo waxed	334	280	282	15.5
Auto. + flexo waxed + N_2 washing	334	262	313	6.3

[1] Carver press: 1,500 lbs. per sq. in.

In further experiments small tubular cellophane casings were used. Into these casings the commercially dehydrated oat material was lightly pressed and the ends merely twisted together and tied with a string. Various treatments were given the material before and after placing in the casings. All of the samples were mixed with water to a content of 15 per cent, giving a total initial moisture content of about 22 per cent. After mixing with the water some of the samples were dried at 95° C. for varying lengths of time. In table 3 the treatments of the materials and the results secured are given.

TABLE 2

Effect of time of autoclaving on preservation of carotene in dehydrated cereal grasses (oats) and alfalfa kept at 22–25° C.

(Pressed into large pellets 3 x 4 in.)

Material and treatment	Carotene content, µg./g. (air-dry basis)		% loss 3 mos.
	Initial	3 mos.	
Dehydrated oats			
Unheated	380	212	44.0
Unheated + flexo wax	380	280	26.0
Unheated + flexo wax + N_2 washing	380	390	00.0
Auto. 60 min.	425	356	16.0
Auto. 60 min. + flexo wax	425	380	10.0
Auto. 60 min. + flexo wax + N_2	413	362	12.0
Auto. 30 min. + flexo wax + N_2	394	399	00.0
Auto. 15 min. + flexo wax + N_2	394	378	4.0
Auto. 5 min. + flexo wax + N_2	382	403	00.0
Dehydrated alfalfa			
Unheated	161	77	52.2
Unheated + flexo wax	161	141	12.4
Unheated + flexo wax + N_2	161	154	4.3
Autoclaved 10 min.			
No wax	147	99	32.6
Flexo waxed	147	131	10.9
Flexo waxed + N_2	147	160	00.0
Autoclaved 30 min.			
No wax	155	105	32.2
Flexo waxed	155	129	16.7
Flexo waxed + N_2	155	166	00.0

The results indicate a loss of carotene where the material was loose although washed with nitrogen and waxed. The result secured with sample no. 2 (table 3) was striking. No loss occurred when the material was unheated and only waxed but contained total moisture at a level of about 20 per cent. Further, the material had turned brown and had a very pleasant aroma. Perhaps the oxygen left in the material had combined with the chlorophyll and the chlorophyll had gone into the "brown" stage, giving an anaerobic

TABLE 3

Effect on carotene preservation of various treatments of dehydrated cereal grasses (oats) involving addition of 15 per cent of water

(Loose in cellophane tubes, kept at 22–25° C.)

Treatment	Color	Carotene content, µg./g. (air-dry basis)		% loss 3 mos.
		Initial	3 mos.	
Unheated—no wax	**Green**	**380**	**189**	**49.7**
Unheated—waxed	**Brown**	**380**	**380**	**0.0**
Unheated—N_2 washed—waxed	**Brown**	**380**	**381**	**0.0**
95° C.—40 min.	**Green**	**418**	**285**	**32.0**
95° C.—40 min.—waxed	**Green**	**418**	**310**	**26.0**
95° C.—40 min.—N_2 washed—waxed	**Green**	**418**	**303**	**27.6**

condition, or possibly the oxygen had been used up by tissue respiration or microorganisms with CO_2 production and O_2 consumption. At any rate, there was complete carotene preservation under a very simple procedure. Where the samples had been dried at 95° C. and the added water lost, the color remained green with considerable loss of carotene.

To secure more data on the behavior of sample 2 (table 3), an extended series of samples was prepared using both commercially dehydrated oats and dehydrated alfalfa.[3] Round cardboard boxes, 3.5 inches in diameter by 4 inches deep, were used as receptacles. The materials were mixed with varying percentages of water, firmly pressed by hand into the receptacles, and then covered tightly. In some cases a 0.5-inch head or air space above the material was left. Some of the alfalfa receptacles were covered completely with a thin layer of wax known as "Durex" and secured from the Dewey and Almy Chemical Company, Cambridge, Massachusetts. After 3 months of storage at room temperature (21–30° C.) the receptacles were opened and carotene determinations made. The results are shown in table 4.

TABLE 4

Effect of varying levels of added water on carotene preservation in dehydrated cereal grasses (oats) and alfalfa kept at 22–25° C.

Treatment	Color	Carotene content, µg./g. (corrected to original water basis)		% loss 3 mos.
		Initial	3 mos.	
Dehydrated oats—10.1 per cent initial water content				
No H_2O added—no wax	Green	379	278	26.6
No H_2O—waxed	Green	379	297	21.6
5% H_2O—waxed	Brown	379	386	00.0
10% H_2O—waxed	Brown	379	431	00.0
15% H_2O—waxed	Brown	379	387	00.0
15% H_2O—½″ head—waxed	Brown	379	410	00.0
Dehydrated alfalfa—7.2 per cent initial water content				
No H_2O added—no wax	Green	159	108	32.0
No H_2O added—waxed	Green	159	142	10.7
5% H_2O—waxed	Brown	159	153	3.7
10% H_2O—waxed	Brown	159	171	00.0
20% H_2O—waxed	Brown	159	171	00.0
20% H_2O—½″ head—waxed	Brown	159	174	00.0
30% H_2O—waxed	Brown	159	169	00.0

All of the materials to which 5 per cent or more of water had been added turned brown in color. Those with the higher levels of water were deeper brown. The added water was in addition to that already present in the material, which was about 7 per cent in the alfalfa and 10 per cent in the oats. Where the color of the product was brown, a pleasant aroma not unlike well-cured silage had developed. In all cases where 5 per cent or more of water had been added and the package covered with a wax to exclude free exchange of gases, the carotene was conserved completely for 3 months. There is no reason to believe that a longer time would have changed the results in this series.

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[3] We are grateful to the Cerophyl Laboratories, Inc., Kansas City, Missouri, for the supply of dehydrated alfalfa and cereal grasses.

There was no bulging of the boxes or evidence of internal gas pressure. However, there may have been a slow diffusion. The nature of the chemical changes and character of the gases produced (if any) were studied further. Where no water had been added, the material remained green in color but a considerable loss of carotene (20–30 per cent) had occurred. In many cases the amounts recorded as carotene were above the initial determinations. This phenomenon has been observed in studies on AIV silage (4) and on the effect of acids on carotenoids (5), and is attributed to the action of the acid on certain carotenoids with production of pigments of similar solubility to carotene. The amount of such pigments produced is relatively small. The fact that pigments of non-carotene nature may be produced by the action of acid on xanthophyll led to an examination of these samples for acidity. Surprisingly, the pH in all of the oat samples, including those where no change in color was observed, was 6.0. In the alfalfa samples it was 6.0–6.3. The higher figure was obtained with the alfalfa to which no water had been added. These figures represent an exceedingly low degree of free acidity.

The pH was determined by suspending 3 g. of the material in 25 cc. of water, stirring, and, after standing 20 minutes, reading on a pH meter. If there were acids produced the amounts must have been quite alike in all samples, mainly, CO_2. Even where a 0.5-inch head of air had been left in the receptacle, no loss of carotene occurred.

Further, to make certain that the observed increase in carotene did not represent an actual loss of carotene, with compensation by formation of other pigments, the carotene content was redetermined by the chromatogram. The method used was that outlined by Wilkes (6). The initial analysis of the alfalfa by the phasic method showed 159 μg. g. After storage for 4 months in the cold room (−4° C.), it showed 143 μg. by the same method. By the chromatographic method 150 μg. of carotene were recovered where 5 per cent of water had been added and 138 μg. where 10 per cent of water was added and the materials kept at room temperature and sealed with flexo wax.

Similar results were secured with dehydrated oats where comparisons were made by the phasic and chromatographic methods. The original analysis of the dehydrated oats showed 379 μg./g. by the phasic method. After 4 months storage in the cold room (−4° C.), it showed 385 μg. by the same method and 344 μg. by the chromatographic method. The experimental samples with 5 per cent of water, sealed with flexo wax and held at room temperature, showed 350 μg./g. by the chromatographic method and, where 15 per cent of water had been added and the samples likewise flexo-waxed and held at room temperature, 360 μg./g. were obtained. If the thesis is accepted that by the chromatographic method more precise data are secured for the carotene content of a sample of dehydrated alfalfa or cereal grass than by the phasic method, then these data confirm the conclusion that practically no carotene is lost by the process of storage outlined. The increases observed under special storage with addition of water may be due, in part, to new pigments and also to limitations of the analytical methods.

To determine whether microorganisms were principally concerned in the reactions observed, samples of dehydrated oats and alfalfa were mixed with 10 per cent of water, placed in pasteboard containers 3.5 × 4 inches, lightly pressed by hand, and sealed with flexo wax. Controls with no additional water also were prepared. These samples were allowed to stand at room temperature for 5 days, then opened and assayed for bacterial count. The results follow:

	Color	*Aroma*	*Bacterial count/g.*
Dehydrated oats, control	No change	No change	2×10^4
Dehydrated oats, +10% water	"	Aroma more prominent	2×10^4
Dehydrated alfalfa, control	"	No change	6×10^4
Dehydrated alfalfa, +10% water	"	Aroma more prominent	4×10^4

The bacteria present were mostly aerobic spore-bearing bacilli, *Bacillus subtilis*. Very few staphylococci and very few mold spores were present.[4] The data indicate that the changes in the material containing added water were not primarily of bacterial origin, at least not in the first 5 days of the experiment.

It was important to determine whether carbon dioxide was being produced and oxygen used up where these dehydrated materials were mixed with added water, and further, whether the changes were occurring in the first 5 days after preparation. To this end 240 g. of dehydrated alfalfa as control (7.5 per cent water) and 240 g. with 10 per cent of water added were lightly packed in separate glass tubes, fitted with proper carbon dioxide guards, and set aside for 5 days at room temperature. At the end of this time the gases in the tubes were swept through weighed potash bulbs and the carbon dioxide determined. The control showed 8.4 mg. CO_2 while the alfalfa containing extra water showed 53.5 mg. CO_2.

In a somewhat similar experiment apparatus was designed to determine the amount of carbon dioxide and oxygen left in the air in contact with the alfalfa mass after standing at room temperature for 1, 3, 5, and 10 days. These experiments were conducted in glass tubes, where contact with outside air was completely nullified. The air-dried material contained 7.5 per cent of water.

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[4] We are indebted to Mrs. M. I. Robblee for this bacterial examination.

The results follow and are expressed in volume per cent of CO_2 and O_2 left in the atmosphere surrounding the alfalfa particles.

	CO_2	O_2
1 day	2.4	17.8
3 days	3.8	16.7
5 days	4.0	14.3
10 days	6.9	10.4

It is evident from the data that the process of respiration was comparatively slow, but was, nevertheless, a condition under which the carotene would be preserved. Just how complete a displacement of the O_2 with CO_2 is necessary for carotene preservation has not been determined.

Dehydrated plant tissue will vary in the rate of respiration, depending upon the temperature and time of drying, as illustrated in the following experiment. Alfalfa, cut on a University field, was spread on the laboratory floor and dried before a fan at a temperature of 22–25° C. This material was ground, lightly packed in glass tubes with and without added water (10 per cent), and the amount of CO_2 determined after standing 5 days at room temperature. The amount of material used in each experiment was 220 g. and contained an initial water content of 12.4 per cent. The air-dry sample (12.4 per cent water) produced 45 mg. of CO_2. The air-dry sample plus 10 per cent of water produced 144 mg. of CO_2. The amount of CO_2 produced where the water had been added represented, approximately, a concentration of 16 per cent of CO_2 in the gas mixture surrounding the alfalfa particles. It is apparent that this material had a respiratory rate appreciably greater than the commercially dehydrated product.

To determine what temperature changes would occur when the dehydrated products were stored with 10 per cent of added water, each of two large fiber cartons lined with paraffin paper was filled with approximately 28 lbs. of dehydrated alfalfa or dehydrated rye and sealed at the cover joint with paper. The material was lightly pressed into the cartons. Thermometers were inserted through the covers and sealed to prevent leakage of air. These cartons were held at room temperature of approximately 23.5° C. There was no observable temperature rise in either carton. Daily readings remained the same and at the end of 2 weeks both cartons recorded temperatures of 23.5° C., which was the room temperature. Theoretically, heat must have been a product of the respiratory changes in these masses but, apparently, the rate of production was so slow that through radiation the constant temperature prevailing in the room was maintained in the mass of material.

Smaller cartons filled with dehydrated alfalfa or rye (after addition of 10 per cent of water) and completely sealed with flexo wax, likewise showed no rise in temperature by the method used.

The data on gas production, bacterial activity, and temperature records lend credence to the belief that tissue respiration is restored after addition of the water. In the dehydration process, not all, if any, of the respiratory enzymes were destroyed and, in the presence of added water and room temperature, their action was renewed. Their activity automatically creates an atmosphere of carbon dioxide with reduced oxygen tension, an ideal condition for carotene preservation.

DISCUSSION

The results secured in these studies emphasize the necessity of oxygen exclusion for carotene preservation in dehydrated cereal grasses or dehydrated alfalfa during storage. Destruction of the lipoxidase by autoclaving without further protection from access to air will not preserve the carotene.

The results secured by mere addition of water to the dehydrated material and then protecting against free access to air seem to offer practical procedures for carotene preservation in these materials. A total water content of 12 to 20 per cent, brought about by the addition of only 5 to 10 per cent of water, and the further protection against free access to air effectively stabilized the carotene for 3 months. In this process the material turned slightly brown and developed a very pleasant aroma. There may be objections to the use of a process where the green color partially is lost, but it should be emphasized that greenness is not always an assurance of a high nutritive value in these materials. At the present time there is no conclusive evidence that cholorophyll has any function in animal nutrition.

Whether the material could be prepared commercially with a final water content of 12–20 per cent has not been studied. Possible difficulties in grinding to a fine state would be encountered. If these materials could be so prepared and then stored under slight pressure in metal or fiber cartons or other containers so constructed as to prevent free exchange of air, it would seem probable that the carotene loss could be reduced considerably, if not prevented entirely. Such a process would save the adding of water after drying the material to a 6–10 per cent water content. The material to which only 5 per cent of water had been added was not so deep brown in color as when 10 per cent or more of water had been added. In fact, an olive green more nearly would describe the color. The nature of the chemical changes is not entirely clear. Oxygen absorption by the chlorophyll, with changes to the brown stage, appears possible but was unconfirmed experimentally. Acid products of a fixable character, such as lactic or acetic, must have been small in amounts and have served only as temporary intermediates.

Samples set up with added water and sealed from free access of air produced estimable quantities of carbon dioxide. Probably the reactions resulting from the addition of the water were those of tissue respiration, with utilization of the oxygen and production of carbon dioxide thus establishing partial, if not complete, anaerobic conditions. Under such conditions it would be expected that the carotene could be preserved.

Bacterial multiplication that could account for the chemical changes produced was not observed. The samples with added water showed no increase in bacterial numbers as compared with the controls in the first 5 days of the experiment; yet carbon dioxide was being produced. It seems probable that the phenomenon observed was one of restored tissue respiration.

Practical application of this principle can be made by the use of containers allowing little or no diffusion of air or carbon dioxide.

It is possible, indeed probable, that the respiratory enzymes left in the dehydrated materials will vary with the temperature and the time the material has been exposed in the process of drying. If this is true, the standardization of the drying method is necessary in order that a maximum of respiratory enzymes survive the process of drying and thus make possible the conservation of carotene by the method outlined.

SUMMARY

1. Destruction of the lipoxidase by autoclaving for 1 hour at 15 lbs. pressure did not preserve the carotene content of dehydrated alfalfa or cereal grasses exposed to air at room temperature.

2. The addition of 5 to 10 per cent of water to these dehydrated materials and then lightly packing them in receptacles sealed with flexo wax or Durex wax preserved the carotene completely for 3 months, when held at room temperature (22–25° C.). The total water content for the oats was 15–20 per cent and for the alfalfa 12–17 per cent.

3. The process of preservation appears to be a restoration of more rapid respiratory enzyme action with utilization of the oxygen and formation of carbon dioxide. There was no indication that bacterial action directly was responsible for the changes that occurred. Temperature changes during the process of respiration were negligible in the masses of material used.

4. It is evident that receptacles with minimum or no air and CO_2 effusion rates are necessary for success in the preservation of the carotene by the method outlined. Oxygen must be excluded or at least held at a low concentration. This phase of the problem is under further study.

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EFFECTS OF SHADE AND SPRINKLING WITH WATER ON SUMMER COMFORT OF JERSEY COWS

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Results from a previous Louisiana test (7) have shown that dairy cows during warm weather spend a large portion of the daytime in the shade and that grazing time between morning and evening milkings may average less than 2 hours. After entering the shade of trees (average time 9:20 a.m.) respiration rates and body temperatures of cows showed a slow but gradual increase, with a maximum registered at 3:00 p.m., when cows entered the milking barn.

As explained by Rhoad (4), cows eliminate body heat by radiation and conduction of heat from their skin, and as latent heat of water vapor from skin and lungs. The portion of elimination by way of the lungs grows in importance as respiration increases, which in turn is most often caused by a rise in air temperature. At 71° F. Forbes, Braman, and Kriss (1) found that about 40 per cent of the heat left the cow's body as latent heat of water vapor. As reported by Kendall (3), the amount of water lost in this manner may vary as much as 12 lbs. per animal daily even when air temperature, feed, and other conditions are kept as uniform as possible. Each pound of moisture lost carries with it 1,086 B.T.U. of heat, he reports, but as air temperature drops there is a decrease in the amount of water eliminated by insensible perspiration.

Rhoad in his review (6) and report on experimental work (5) shows how time spent by cattle in the shade is associated with heat tolerance and that those with low tolerance, such as Angus, spend much more time in shade than do those of high tolerance, such as Brahman. He found Jersey cattle comparable to crossbreds carrying one fourth Brahman and three fourths Angus blood. Tests with these crossbreds on a hot day (air temperature 80–102° F.) showed a respiration rate of approximately 90 per minute while in the sun, with a drop to around 40 after 1 hour in the shade. Body temperatures were approximately 102.8° F. and 101.4° F., respectively, under these two conditions. Tests on a cooler day, with air temperature between 80 and 84° F., showed less change due to shade, *i.e.*, respiration rate was around 50 while cattle were in the sun, but dropped to 30 after 1 hour in the shade. Body temperature changed from approximately 101.7° F. in the sun to 101.0° F. in the shade.

Reports by Villegas (10) on use of an air-conditioned barn near Singapore with temperature kept at 70° F. showed Holstein cows averaging 24 lbs.

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of milk daily as compared to 9 lbs. from cows in an open, well-ventilated barn exposed to tropical temperatures. Reproduction records showed that 58 per cent of cows in the air-conditioned barn conceived as compared to 25 per cent in the other barn.

Conversation with people who have visited the tropics has disclosed observations made by them where water in a pond or stream or from various water sprinkling devices was used to make milking animals more comfortable during hot days. This applies particularly to water buffalo and to a lesser extent to cattle. Observations in Louisiana (9) revealed that Holstein cows had body temperatures on warm days that averaged approximately 0.75° F. higher than did Jerseys within the same herd. A high percentage of these Holsteins sought relief by lying in water and mud whenever available, a practice not often followed by the Jersey cows.

EXPERIMENTAL

Four grade Jersey cows were observed over a period of 10 days in an attempt to determine how shade alone, or shade following sprinkling with water, affected milking cows removed from the effects of direct sun's rays during the summer. Bright sunshiny days were selected for making the tests; the first day of observation was on June 22, 1945, and the last one on August 1, 1945. Air temperatures in the shade varied from 83 to 90° F. and relative humidity from 61 to 80 per cent.

The reversal experimental design was used for the test. On each test day the cows were tied by halters to a fence located in the sunshine. They were left exposed to the direct rays of the sun between the hours of 12:00 noon and 2:00 p.m. Records then were made of rectal body temperatures, respirations as indicated by flank movements, and pulse rates as determined by placing tips of fingers on the underside of the tail and adjacent to the coccygeal artery. Following this, two of the cows were removed (dry) to the shade of a small barn (with numerous openings for ventilation) while the other two first were sprinkled thoroughly with water varying from 83 to 85° F. before being taken into the barn. The procedure was varied so that each pair of cows had 5 days when they entered shade without sprinkling (dry) and 5 days when they were sprinkled first before entering the shade of the barn. Sprinkling was performed by using a hand-type sprayer and thoroughly wetting all portions of the cow's body. Records were made of body temperature, pulse rate, and respiration rate 0.5 hour and 1 hour after cows entered the shade of the barn, using the same procedure as when cows were outside.

RESULTS

Body temperature. In each case shade alone, as provided by the barn, was effective in reducing materially the body temperatures of the dry cows. Mean reduction for 5-day records on individual cows after 0.5 hour in shade (table 1) varied from 0.24 to 0.42° F. with an average of 0.34° F. After remaining in the shade 1 hour, the reductions varied from 0.58 to 0.88° F., with an average of 0.74° F. Thus, actual body temperature after 1 hour in the shade averaged 101.92° F. This was lower than after 0.5 hour by 0.4° F. Reductions after 1 hour were 118 per cent greater than at the half-hour period.

When cows were sprinkled prior to entering the shade of the barn, their mean body temperature reductions in 0.5 hour varied from 0.3 to 0.8° F. with an average of 0.54° F. After 1 hour in the shade the mean reduction for the four cows varied from 0.78 to 1.4° F. In this case the 1-hour-period decreases averaged 1.08° F., or 100 per cent greater than at the end of 0.5 hour.

TABLE 1

Changes in body temperature caused by shade or shade plus water sprinkling
(5-day mean values for individual cows)

Cow no.	Shade alone trial			Sprinkling plus shade trial		
	Body temp. after 2 hr. in sun	Body temperature reduction		Body temp. after 2 hr. in sun	Body temperature reduction	
		After 0.5 hr. in shade	After 1 hr. in shade		Wet and in shade 0.5 hr.	Wet and in shade 1 hr.
	(°*F.*)	(°*F.*)	(°*F.*)	(°*F.*)	(°*F.*)	(°*F.*)
3	102.46	0.26	0.70	102.14	0.30	0.78
4	102.42	0.42	0.82	102.20	0.54	1.00
11	103.24	0.42	0.88	103.22	0.80	1.40
12	102.50	0.24	0.58	102.56	0.54	1.16
Av.	102.66	0.34	0.74	102.53	0.54	1.08

Cows sprinkled prior to entering shade averaged at the end of 0.5 hour 0.2° F. lower than when not sprinkled; and after 1 hour they were 0.34° F. lower. The advantage of sprinkling vs. not sprinkling at the end of 0.5 hour was 59 per cent, and at the end of 1 hour 46 per cent. Actual temperatures of sprinkled cows after 1 hour in the shade averaged 101.45° F., or well within the range of normal (2).

Respiration rate. After being in the shade (dry) for 0.5 hour, respiration rates reduced from an average of 83 to 55.8 per minute. Actual mean reductions due to shade alone (table 2) ranged from 22.6 to 32.6 among the four cows and averaged 27.2. With but one exception (cow no. 12), respiration rates were slightly faster at the end of one hour than at the half-hour period. The average reduction was 25.2 per minute, showing that cows were breathing two respirations per minute faster than at the half-hour period.

Cows when sprinkled prior to entering shade dropped much lower in respiration rates than when not sprinkled. Before sprinkling, respiration

TABLE 2

Changes in respiration rate caused by shade or shade plus water sprinkling
(5-day mean values for individual cows)

Cow no.	Shade alone trial			Sprinkling plus shade trial		
	Respiration after 2 hr. in sun	Respiration reduction		Respiration after 2 hr. in sun	Respiration reduction	
		After 0.5 hr. in shade	After 1 hr. in shade		Wet and in shade 0.5 hr.	Wet and in shade 1 hr.
	(per min.)	*(per min.)*	*(per min.)*	*(per min.)*	*(per min.)*	*(per min.)*
3	74.0	22.6	16.5	74.0	37.8	34.2
4	88.2	26.4	22.2	82.2	39.8	36.6
11	80.8	27.4	24.4	92.2	50.8	37.2
12	89.2	32.6	37.8	105.6	69.2	58.6
Av.	83.0	27.2	25.2	88.2	49.4	41.6

rates averaged 88.2 (table 2), while at end of 0.5 hour in shade they averaged 38.8, a reduction of 49.4. At the 1-hour period respirations had increased to an average of 46.6 or 7.8 per minute faster than at end of 0.5 hour. When compared to reductions in respiration rates of non-sprinkled cows, the reductions following sprinkling were 81 per cent greater at the half-hour period and 65 per cent greater at the hour period.

Pulse rates. Pulse rates appeared to change more slowly than did body temperatures or respiration rates. When dry cows entered the shade, they showed little change in pulse rate at the end of 0.5 hour (table 3). The average reduction from the original rate of 68.5 was only 0.8 per minute. Cow no. 11 actually averaged faster by 0.6 per minute. Shade alone at end of 1 hour effected a significant change in pulse rate, with mean reductions for cows varying from 1.0 to 5.8 and averaging 3.6 per minute.

Sprinkling of cows prior to entering shade resulted in a great reduction in pulse rate. After 0.5 hour in shade pulse rates had dropped an average

TABLE 3

Changes in pulse rate caused by shade or shade plus water sprinkling
(5-day mean values for individual cows)

Cow no.	Shade alone trial			Sprinkling plus shade trial		
	Pulse rate after 2 hr. in sun	Pulse rate reduction		Pulse rate after 2 hr. in sun	Pulse rate reduction	
		After 0.5 hr. in shade	After 1 hr. in shade		Wet and in shade 0.5 hr.	Wet and in shade 1 hr.
	(per min.)	*(per min.)*	*(per min.)*	*(per min.)*	*(per min.)*	*(per min.)*
3	71.8	1.8	5.8	67.6	7.8	7.6
4	69.0	0.6	1.0	64.8	5.8	3.8
11	69.6	−0.6	5.8	71.0	2.8	7.0
12	63.6	1.2	2.0	68.6	8.0	7.9
Av.	68.5	0.8	3.6	68.0	6.1	6.6

of 6.1 per minute, and the reduction averaged 6.6 per minute 1 hour after entering shade, leaving actual pulse rate at 61.4 per minute, the lowest of any observation period.

DISCUSSION

In the present study cows entering shade (dry) derived more benefit from the shade furnished by the openly ventilated barn than did cows observed in a previous study (7) utilizing shade of trees in a pasture. It is probable that the barn furnished more complete shade than did the trees in the pasture. Also, air temperatures in the previous study continued to increase after cows entered shade (average time 9 : 20 a.m.) ; in this study there was little increase in air temperature after 2 : 00 p.m., when cows were taken from sunshine into shade. Another factor to consider is that cows used in the present study were all grade Jerseys producing only small amounts of milk. In the pasture study (7) heavier producing cows were observed and one-half of the cows were Holsteins, a breed shown (9) to have a lower rating on heat tolerance than Jerseys. It is possible that a higher humidity in the forenoon than in the afternoon (which is usual in Louisiana) may be a contributing factor to differences found in these two experiments. The effects due to this cause, however, would not be expected to be large, in view of results from a previous study (8), where it was found that high humidity played a minor rôle as a factor affecting body temperature, respiration rate, and pulse rate of dairy cows.

When cows in this study were sprinkled with water prior to entering shade, their body temperatures and respiration rates rapidly approached what has been reported as normal (2). It probably would require periodic sprinkling, perhaps once per hour, to hold such cows closer to normal than cows going into shade without sprinkling. However, as has been shown in this experiment, the unsprinkled group of cows would be slower in approaching normal. Whether or not practical sprinkling devices can be developed remains to be seen. Preliminary trials by the authors have shown that cows do not care to go into a coarse spray of water such as that produced by a conventional hose nozzle often used for lawns and gardens. Cows will go into a finer spray when located in the shade, according to a verbal report from a dairyman having had experience on a tropical island during the last war emergency period. Likewise, it has been observed by the authors that cattle when abnormally warm will usually relish wading into streams or ponds, particularly if they are located in the shade.

SUMMARY

1. Four grade Jersey cows were observed during 10 relatively warm days in an effort to determine how shade alone or sprinkling with water followed by shade affected their comfort. Air temperatures during periods of obser-

vation varied from 83 to 90° F. and relative humidity between 61 and 80 per cent.

2. Body temperatures of cows after exposure to sunshine for 2 hours averaged 102.66° F., and removal to shade (dry) resulted in reductions of 0.34° and 0.74° F. after 0.5 hour and 1 hour, respectively.

3. Sprinkling of cows (with original body temperatures after being in the sun of 102.53° F.) reduced body temperatures by 0.54° F. after 0.5 hour in shade, and by 1.08° F. after 1 hour in shade. In the latter case cows had temperatures which are considered normal.

4. Respiration rates reduced to levels which averaged lower after 0.5 hour than after 1 hour in shade. Cows not sprinkled showed a respiration rate of 83 per minute in the sun and an average decrease in rate of breathing of 27.2 at end of 0.5 hour and 25.2 after 1 hour in the shade.

5. Cows sprinkled prior to entering shade had much greater reductions in rate of breathing than did non-sprinkled cows, *i.e.*, 49.4 less after 0.5 hour and 41.6 less after 1 hour.

6. Reductions in rate of breathing for non-sprinkled cows vs. those sprinkled favored the latter group by 81 per cent at end of 0.5 hour and 65 per cent after 1 hour in shade.

7. Average reduction in pulse rate for non-sprinkled cows after being in shade 0.5 hour was insignificant (0.8 per minute) but was significant (3.6) after 1 hour. Cows when sprinkled showed decreases in pulse rate that averaged 6.1 after 0.5 hour and 6.6 after 1 hour.

8. Either shade alone or sprinkling followed by shade was found effective in reducing body temperature, respiration rate, and pulse rate of dairy cows, with the second procedure being more rapid and also more effective in causing animals to approach readings which are considered normal.

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ETHYL ALCOHOL FROM WHEY

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The preliminary report by Browne (1) on the production of ethyl alcohol from the fermentation of lactose in whey was published before the War. This early work with small batches of material gave evidence of promise. Whey as a raw material seemed a likely source of alcohol, since it probably is as cheap as any source of fermentable sugar, if a sufficient supply is readily and locally available. Furthermore, there is the constant problem, sometimes serious, of reducing the B.O.D. of effluents to streams. Therefore, work was conducted on the selection of desirable yeast strains, on the physiology of lactose-fermenting yeasts, of which practically nothing was known, and on the definition of the conditions for a more efficient and economically feasible fermentation of lactose to alcohol by yeast.

EXPERIMENTAL AND RESULTS

At the beginning of this work it was quickly apparent from the results being obtained with the very few strains of lactose-fermenting yeasts then available, that it was essential to examine many different types of lactose-fermenting yeasts for their suitability for alcohol production. Accordingly, a large and heterogeneous collection of lactose-fermenting yeasts was acquired.

The ability of the different types of lactose-fermenting yeasts to ferment the lactose in whey was measured by direct analyses of the residual lactose in the fermenting flasks after various periods of incubation at the optimal fermentation temperature for each organism. In each instance at least five analyses were made at different times during the course of a fermentation period. The relative rates of fermentation of whey containing 5 per cent of lactose at the beginning of the fermentation are shown in figure 1. After 55 hours *Torula cremoris* #2 had fermented all the lactose. In comparison, the following percentages of residual lactose were present in the case of other organisms tested: *Zygosaccharomyces lactis*, 3.8; *Torulopsis kefir*, 2.9; *Mycotorula lactis*, 3.1; *Candida pseudotropicalis*, 1.7; *Saccharomyces anamensis*, 1.6; *Saccharomyces lactis*, 1.7; *Saccharomyces fragilis*, 1.4; *Torula lactosa*, 1.3; *Torula sphaerica*, 1.2; and Type F, an unidentified lactose-fermenting yeast, 1.3. Thus, certain strains of *Torula cremoris* were the most efficient of all the tested lactose-fermenting yeasts in the fermentation of lactose in whey. Consequently, the later pilot plant experiments were conducted with a strain of this yeast, *Torula cremoris* #2.

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[1] Deceased January 7, 1947.

It then seemed advisable to determine the optimal conditions for fermentation. Tests at temperatures of 30°, 32°, 34°, 37° and 42° C. were made in a number of experiments, the results of which are depicted in figure 2. Fermentation took place faster at 37° C. than at any of the other temperatures used. However, after slightly longer intervals of time than are shown in the figure, the initial and somewhat later superiority of 37° C. over 32° C. is hardly apparent, and usually there is very little difference in the times necessary to effect complete fermentation (no residual lactose) at the two temperatures. Also, in fermenting larger batches of whey (150 gallons) which had an initial temperature of 30° C., the temperature rose to a maximum of 33–34° C. (heat of fermentation) and remained at this level throughout the most active period of the fermentation. Because of these considerations and also because higher temperatures may induce greater losses of

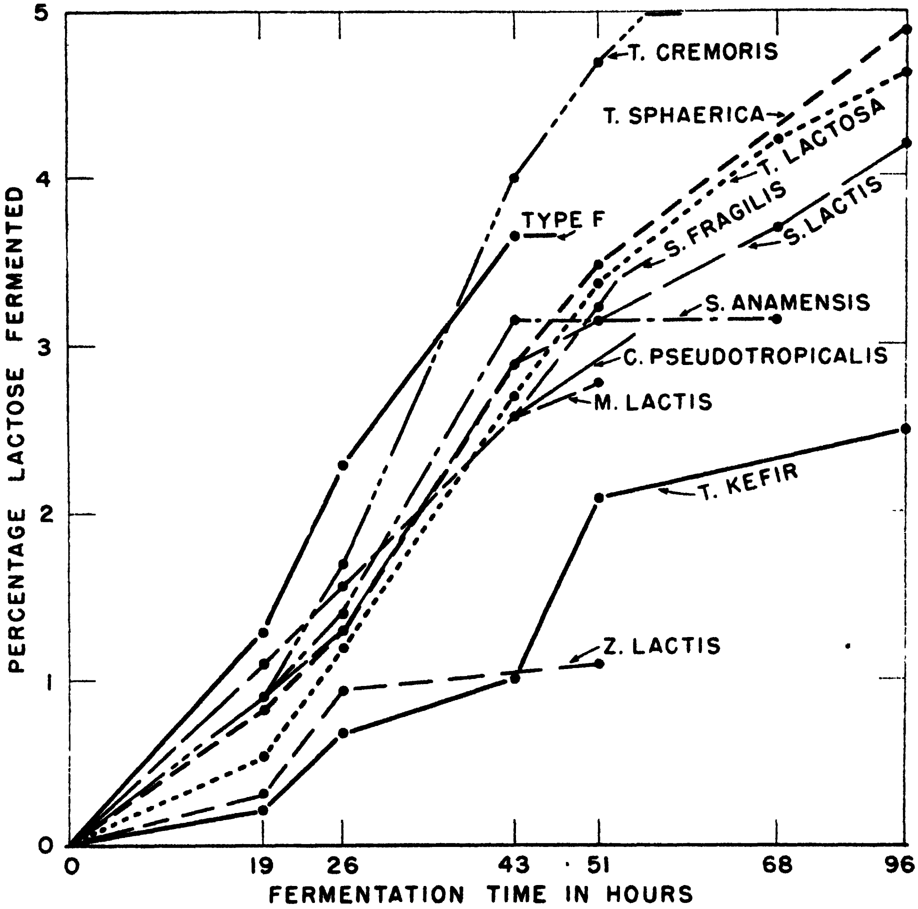

FIG. 1. Relative efficiency of lactose fermentation by lactose-fermenting yeasts.

alcohol by evaporation, thus lowering the yield, it is recommended that a temperature range of 33–34° C. be used.

The question as to how much yeast should be used in the fermentation is important in terms of the time and economy of the fermentation. On the basis of the average number of grams of lactose fermented per gram of yeast per hour of elapsed fermentation time, it was established that a maximum amount of yeast corresponding to 2 per cent of the weight of the lactose initially present is sufficient to ensure a satisfactory rate of fermentation. In experiments with larger batches of whey, amounts of yeast as low as 1 per cent fermented the whey at a satisfactory rate if the yeast was in good condition.

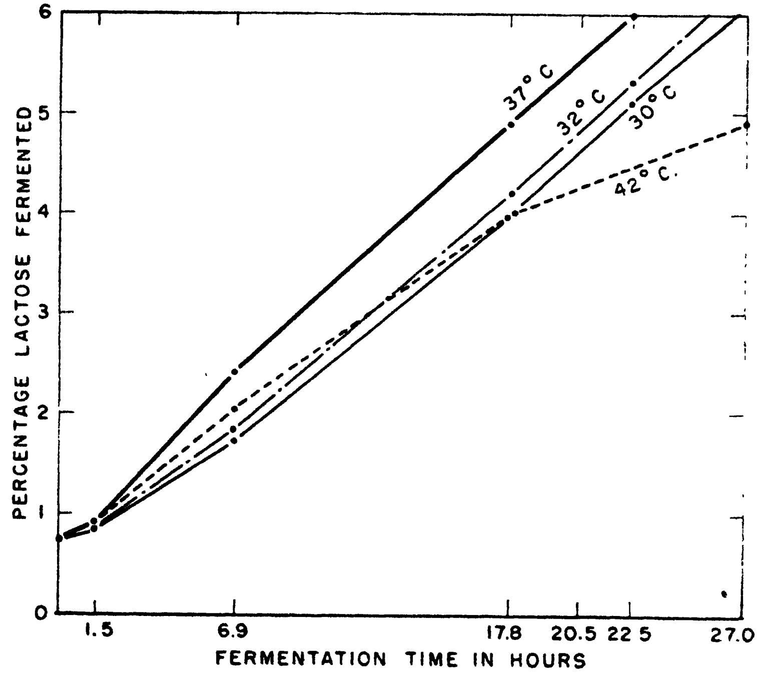

FIG. 2. Influence of temperature on the rate of lactose fermentation by *Torula cremoris*.

The whey may be treated with heat, and either sour whey or acid may be added to precipitate the protein in the whey before the fermentation. If the whey is not treated, the initial pH of the whey mash should approximate a value of 6.0. If the whey is acidified, our experience with different batches and types of whey has been that the initial pH of the clarified whey should lie within a range of 4.8 to 5.2. Obviously, it is important to know what effects the initial pH, as well as the change in pH during the course of the fermentation, have on the rate and extent of lactose fermentation by the yeast. Also, it is desirable to conduct the fermentation at as low a pH value as possible in order to minimize growth of contaminating microorganisms. Clarification may be of great advantage regardless of the utility or economy of isolating the whey protein, since the pH is lowered.

Results of fermentation begun at different pH levels from 6.0 to 4.6 are depicted in figure 3. A pH range of 4.7 to 5.0 is satisfactory for a good fermentation. Peculiarly, an initial pH of 6.0 (unclarified whey) also was satisfactory, whereas at intermediate pH levels irregular and less satisfactory results were obtained.

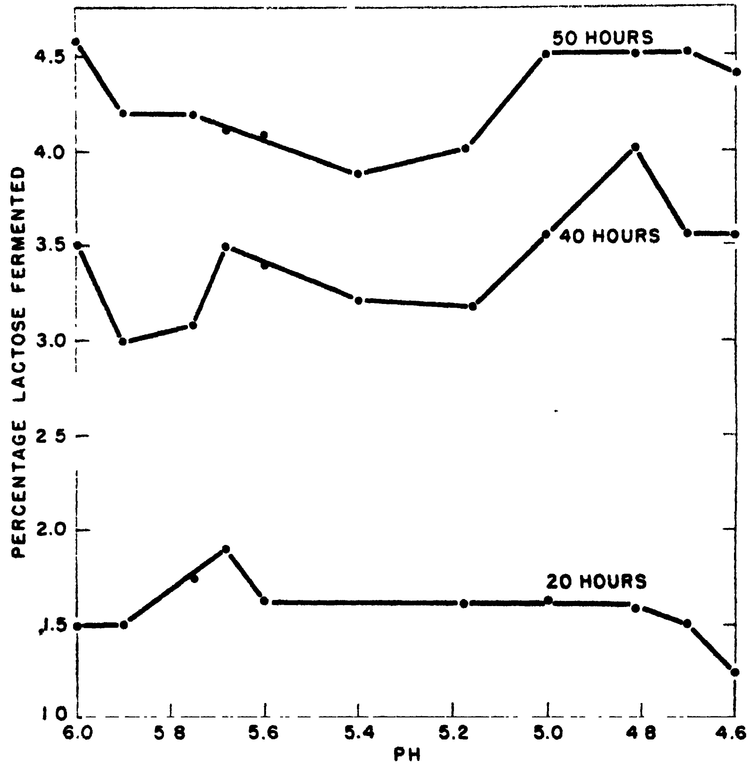

FIG. 3. Influence of pH on the fermentation of lactose by *Torula cremoris.*

The course of pH change from the initial value is shown in figure 4. A relatively small change in acidity occurs during the course of a fermentation when the fermentation is begun within the range of pH 4.7 to 5.0. In view of these results, it is recommended that the initial pH of a whey mash be adjusted to a range of 4.7 to 5.0.

Yields averaging 90.73 per cent of the lactose as alcohol have been obtained from the complete fermentation on a laboratory scale. Under semi-plant conditions yields were somewhat lower (as low as 84 per cent), probably due to the inefficient still employed. These yields compare favorably with other processes.

The quality of the alcohol was highly satisfactory. Customary "rub" tests for fusel oils and esterification tests for amyl alcohol were negative. Like other alcohols produced from grain and similar raw materials, this alcohol contains small quanties of aldehydes. However, they may be eliminated conveniently in the rectification of the crude distillate.

By-products from the fermentation, such as the whey protein and the slops, are of value as feed. The riboflavin and other vitamin content will compare well, if not more favorably, with the vitamin content of the original whey on a dry basis. If the slops are to be dried for feed, the beer should be distilled at its naturally acid pH. Subsequent rectification of the crude alcohol can be conducted in the alkaline region to remove aldehydes.

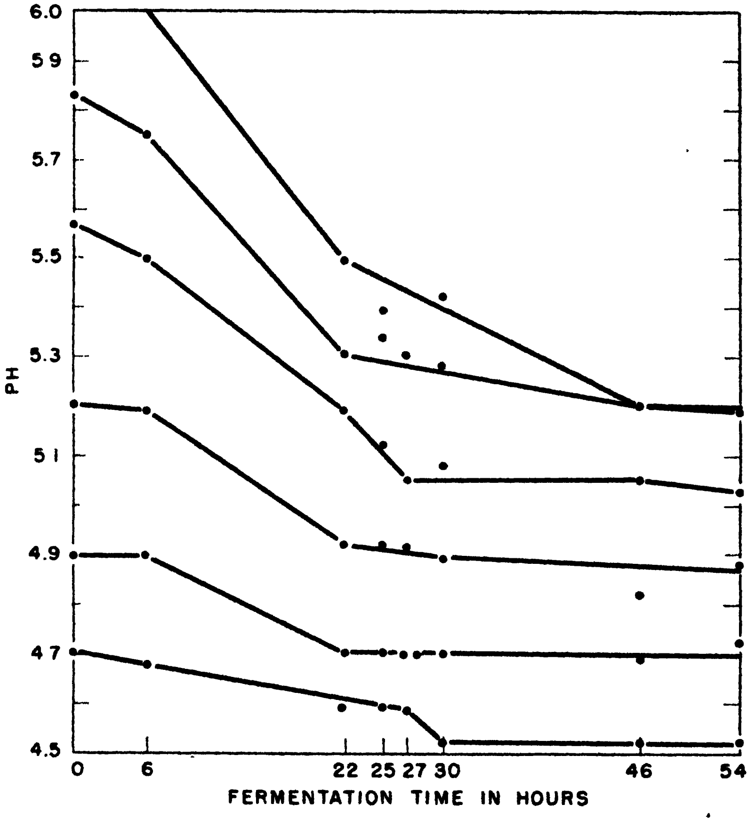

FIG. 4. Course of pH change during fermentation by *Torula cremoris*.

EQUIPMENT AND OPERATION

On the basis of experimental work with 150-gallon batches of whey, it appears that the equipment for alcohol production should include at least the following items: a separator for removing the fat from the whey, a tank fitted with a steam pipe for heating the whey, a filter press, a cooler for cooling the heated whey, one or more closed fermenting vats depending on the capacity of the plant, yeast tubs in a yeast room for propagating the yeast, an air line provided with a filter to furnish sterile air, a yeast recovery separator, an appropriately designed still, a storage tank for the distilled alcohol, condensing and drying facilities for the stillage slops if they are to be recovered, pumps, pipe lines, and a source of steam.

The accompanying flow-sheet (figure 5) gives a simplified and general picture of a total operation. The steps in which a filter press and yeast recovery separator are used may be omitted. This is true particularly if the slops are to be recovered and sold for feed. In this case all the protein in the whey and the yeast from the fermentation are wanted in the condensed or dried stillage slops for their nutritional value.

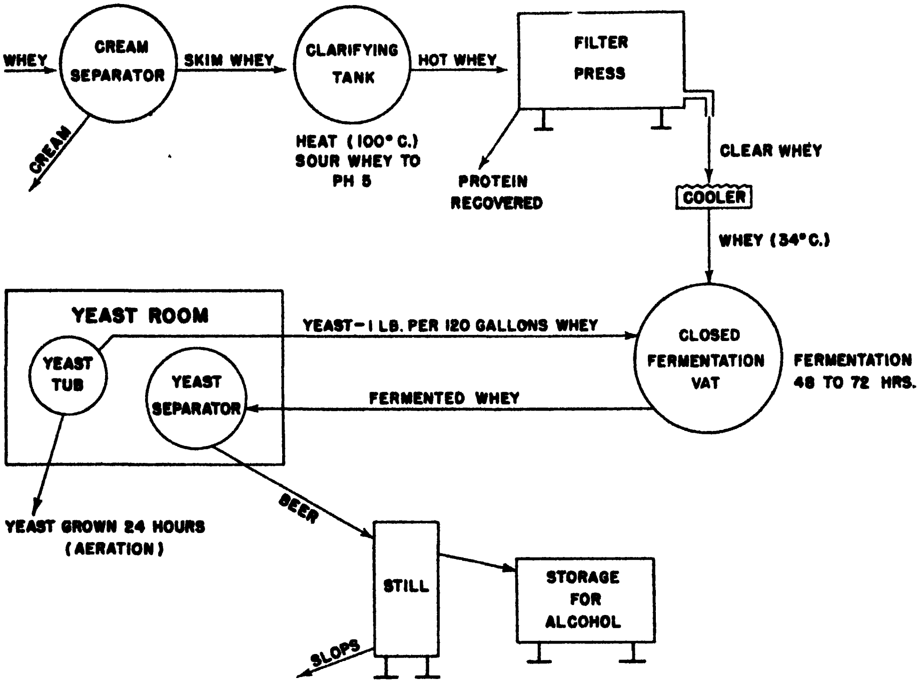

FIG. 5. Flow-sheet of alcohol production from whey.

DISCUSSION

It is difficult to predict the costs involved compared to grain or molasses fermentation because much depends on local conditions, such as availability of raw materials; nevertheless, it is possible to make some tentative statements. Capital costs may compare favorably with other processes, since the alcohol plant could be a by-product plant operating as an adjunct to a cheese or casein factory from which some space, equipment, and low-pressure steam would be available. In this case, the major capital outlay would be limited to the cost of clarifying, fermenting, condensing, and distilling equipment. The steam cost for a finished 95 per cent alcohol from whey would be higher than that from other processes, since a grain or molasses mash can be fermented to a higher alcohol content. But the relative cost of capital outlay, raw materials, and similar items probably would compensate for the higher steam costs of the whey process. However, higher steam costs might be reduced considerably and even to a favorable competitive basis because sugar (glucose sirups or molasses) might be added to the whey and fermented to a higher alcohol content than the lactose naturally present in whey would permit. The addition of other sugars is feasible since whey contains all the necessary growth factors for the fermentation of these sugars by lactose-fermenting yeasts. Since the supply of whey may not be steady, and since the plant should be constructed for a maximum supply of whey, the addition of sugar sirups seems to be an economically desirable feature of the process.

SUMMARY

1. Various types of lactose-fermenting yeast were tested for their efficiency in fermenting lactose in whey, and *Torula cremoris* was selected as the most efficient organism.

2. The optimal operating temperature for the fermentation in the semiplant was found to be 33–34° C.

3. The pH of a whey mash should be within the range of 4.7 to 5.0.

4. Yeast equivalent to two per cent of the weight of the lactose is sufficient for satisfactory fermentation.

5. Yields of alcohol were obtained averaging 90.73 per cent in the laboratory and as low as 84 per cent in the plant.

6. The equipment and the operation of a plant producing alcohol from whey are described.

7. The relative economy of the process is discussed.

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(1) BROWNE, H. H. Ethyl Alcohol from Fermentation of Lactose in Whey. Indus. and Engin. Chem., News Ed., 19: 1271. 1941.

ASSOCIATION ANNOUNCEMENTS

ANNUAL MEETING: ONTARIO AGRICULTURAL COLLEGE, GUELPH, ONTARIO, CANADA, JUNE 24–26, 1947

ABSTRACTS OF PAPERS

All abstracts of papers to be given at the annual meeting must be received by the section Chairman by May 30. They should be mailed to the committee chairman to whom the title was sent.

REGISTRATION AND HOUSING

Registration headquarters will be in the Administration Building, Ontario Agricultural College, Guelph, Ontario.

Housing facilities will be available in College dormitories. Rooms in local hotels are very scarce. Meals will be served cafeteria style in Creelman Hall. An attempt will be made to house family groups in the same dormitories. Rooms and meals will cost $2.50 a day. Individual meals for delegates not in the dormitories will cost 50¢ each. A return card relative to advance registration and housing will be sent to members by the Association Secretary in May.

PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for projection of standard and 2″ × 2″ slides. Projectors for 16-mm. movies will be available by arrangement. Request for projection equipment should be made at the time abstracts of papers are submitted to the respective section Chairman. For the benefit of any bringing special electrical equipment, the available current is all 25 cycle.

COMMITTEE MEETINGS

Those wishing rooms for Extension and Production Section Committee meetings should write or contact G. E. Raithby and those in the Manufacturing Section wishing the use of rooms for Committee meetings should write or contact W. H. Sproule.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings before, during, or after the regular session will please contact G. E. Raithby. Provision can also be made for a limited number of breakfasts, luncheons, or dinners for special groups.

TRAVEL SUGGESTIONS

Guelph is serviced by the two main railway lines in Canada, Canadian National and Canadian Pacific. Bus lines from the South, East and West lead to the city. Highways nos. 6, 7, and 24 pass through the city, and motorists should make inquiry after crossing the border for highway suggestions. Representatives of the Ontario Tourists Association, located at border crossing points in Ontario, will be notified of the Conference and will be glad to give assistance. The nearest airport is at Malton, Ontario, about fifty miles from the College. Malton is on the main line of the Canadian National Railway between Toronto and Guelph.

RECREATION

On the campus are tennis courts, baseball diamonds, and an indoor swimming pool. Adjoining the College is a pay-as-you-play golf course.

G. E. Raithby of the Animal Husbandry Department, Ontario Agricultural College, Guelph, has been designated as the representative of the host institution.

W. D. Tolton of the Ontario Agricultural College, Guelph, has been appointed to membership on the Extension Section Committee on Teaching Methods and Exhibits.

JOURNAL OF DAIRY SCIENCE

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WITHIN-COW REGRESSION OF MILK-ENERGY YIELD ON AGE AND LIVEWEIGHT[1]

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Nebraska Agricultural Experiment Station, Lincoln

INTRODUCTION

Certain DHIA records of cows in Illinois farm herds (3, 4) showed that milk-energy yield is practically unaffected by age of cow, independent of liveweight. On the other hand, milk-energy yield is greatly affected by liveweight, independent of age. These results relate to Holstein and Jersey cows, separately by breed. In each breed some of the cows were registered animals and some were not.

A similar result was found (1) from certain more accurate records (*e.g.*, milk weighed at each milking instead of one day per month) in the Nebraska Station herd at Lincoln. The Nebraska data are for registered cows of the Ayrshire, Guernsey, Holstein, and Jersey breeds, all treated as one group. The lumping of the data for the four breeds quite possibly could disturb the general validity of the result, because the Holsteins were markedly larger than the other breeds and at the same time had a decidedly higher milk-energy yield per unit liveweight than the other breeds. The present paper presents a refinement of the analysis for the Nebraska data by finding the age-weight-yield relation within cow for the Holstein breed.

PROCEDURE AND RESULTS

The general principle of statistical procedure used is the same as that used by Dickerson (2) in finding the within-cow regression of milk-fat yield on age (liveweight ignored). The present procedure involves fitting a three-constant equation to the observations; accordingly, only cows with three or

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[1] **Published with approval of the Director as Paper no. 409, Journal Series, Nebraska Agricultural Experiment Station, and with approval of the Director of the Illinois Station. The original observations were made at the Nebraska Station, and the computations were made at the Illinois Station, utilizing IBM punched-card machines.**

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more lactations are used. The data provide 57 cows, each with 3 or more lactations, a total of 231 lactations distributed as follows:

Lactations	3	4	5	6	7	8	9
Cows	25	15	11	3	2	0	1

The following within-cow, within-breed, within-herd equations emerge, by least squares:

$$W = 917.4 + 9.822\,A - 0.04048\,A^2 \qquad \text{(a)}$$

$$FCM = -1.69 + (0.0413 \pm 0.0045)\,W + (0.001 \pm 0.022)\,A \qquad \text{(b)}$$

$$1{,}000\,FCM/W = 38.30 + (0.0012 \pm 0.0032)\,W + (0.004 \pm 0.016)\,A \qquad \text{(c)}$$

$$1{,}000\,FCM/W = 31.00 + (0.245 \pm 0.044)\,A - (0.00142 \pm 0.00026)\,A^2 \qquad \text{(d)}$$

W is liveweight in pounds, the average of three scale weighings on successive days within 31 days after calving. This particular stage of lactation for the measurement of liveweight is an essential feature of the present philosophy. Class units of one pound were used in the computations.

A is conceptual age of cow at calving in months, reckoned as 10 + birth age at calving in months, with all fractions dropped. The record of birth age at calving is accurate to a day but class units of one month were used in the computations.

FCM is milk-energy yield for the 8-month partial lectation in pounds of 4 per cent milk per day. The 8-month (243-day) partial lactation is used to avoid complications of advanced pregnancy. ***FCM*** is based on milk weights at each milking and monthly determinations of fat percentage. Class units of 0.1 lb. were used for ***FCM*** and 1,000 ***FCM/W*** in the computations.[2]

W, A, FCM, and 1,000 ***FCM/W*** are known for each lactation of each cow, and the equations deal with these items in the same lactation. For example, ***FCM*** is related to *W* of the same lactation, not *W* of some other lactation. The mean and range for the 231 lactations are as follows:

	A	A^2	W	FCM	$1{,}000\,FCM/W$
Mean	72	5,920	1,385	55.6	40.2
Range	35–174	1,225–30,276	979–1,854	31.9–80.7	25.4–56.4

DISCUSSION

Accepting the observations and calculations as of a satisfactory order of accuracy, what biological interpretations are warranted by the equations?

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[2] ***FCM*** = 0.4 × milk + 15 × milk fat, all in the same unit of weight. One pound ***FCM*** = 340 kilocalories of milk energy. The correlation between milk-energy yield in calories determined by use of direct calorimetry and milk-energy yield in calories estimated by use of the ***FCM*** formulas is of the order 0.997. If the milk-yield and fat-yield data are valid, the estimate of milk-energy yield in terms of 4 per cent milk by the ***FCM*** formula also is valid. ***FCM*** is a technic for estimating milk energy. It is not a correction for fat percentage in the same sense that mature equivalent is a correction for age.

In the first place the equations are within cow (and, by physical restriction, within breed and within herd), which should enhance their biological meaning.

By equation (a) $W = 1{,}212$ at $A = 35$, reaches a maximum of 1,513 at $A = 121$, and then declines to 1,401 at $A = 174$. (Birth age is approximately 9.5 months less than A.) Holsteins are a large breed and the present 57 Holsteins are large animals of the breed. The declining phase of equation (a) is not very reliable because of a sparse population at values of A beyond 121. Taken at face value, it may indicate a lack of condition (fatness) at calving at advanced ages. Also, the equation is bound to be affected by the individuality of the few cows having lactations at advanced ages.

Equation (b) expresses *FCM* as a function of W and A. The coefficient of W is nine times as large as its standard error and highly significant in a probability sense. On the other hand, the coefficient of A is smaller than its standard error, *i.e.*, it does not differ significantly from zero. According to equation (b), an increase of 100 lbs. in liveweight is accompanied by an increase of 4.13 in *FCM*, independent of age, which is 344 times as great as the increase in *FCM* which accompanies an increase of one year in age, independent of liveweight. One pound of liveweight is more potent than 3 years in age, by the equation.

This within-cow result is in general agreement with the previous findings (1, 3, 4) that milk-energy yield is influenced profoundly by liveweight, independent of age, and is substantially unaffected by age, independent of liveweight. If these are the true relations, then the system of correcting milk yield for age of cow at calving is biologically unsound and should be superseded by a system based on liveweight within 31 days after calving.

The foregoing statement is not intended to deny the statistical validity of age correction where W is unknown, because age and liveweight are to some extent correlated. In the present study of 231 lactations, the correlation between A and W is 0.48 in total, 0.31 between cows, and 0.62 within cow. But this does not alter the biological situation. Age represents time, which is required for the organism to attain size (liveweight). The procedure of equation (b) is to allow age and liveweight to take their natural values and depend on the liveweight and age terms of the equation, adjusted by least squares to the observations within cow, to segregate the independent influence of age and liveweight. This procedure allows both age and liveweight to express themselves in a natural way. If age is restricted to first lactations, the range of liveweight is restricted because the animals have not had time to grow fully and, of course, the within-cow relationships cannot be ascertained at all.

Equation (c) proceeds to the next logical step, namely, since equation (b) indicates that *FCM* is largely a multiple of W, with the other two terms of little consequence, FCM/W should be largely independent of both age and liveweight. Equation (c) shows that such is the case. The coefficients of W and A are smaller than their respective standard errors. Neither one is significantly different from zero.

Milk-energy yield per 1,000 lbs. liveweight, 1,000 FCM/W, is here regarded as a quantitative measure of lactational drive, or the intensity of lactation metabolism, on the assumption that lactation metabolism is proportional to milk-energy yield and the amount of protoplasm (work stuff) involved in lactation is proportional to liveweight.[3]

FCM/W stands in its own right as a factual measure of lactational drive. It is not to be regarded merely, or primarily, as a substitute for age correction. It is, rather, a direct biological measurement which has no need of age correction. Clearly, the lactational-drive philosophy is very different from the mature-equivalent philosophy.

In a similar way FCM/W is not to be regarded as a correction for weight. It is, again, a factual biological measurement which has no need of weight correction. The evidence substantiating this statement is satisfactory as within breed (Holstein or Jersey) but as yet is not conclusive as between breeds (Holstein and Jersey). When all the evidence is collected, we may find FCM/W is greater for Jerseys than for Holsteins (as FCM/W is unmistakably greater for dairy goats than it is for dairy cows). On the other hand, FCM/W, as used here, may prove to be a biologically equitable measure of lactational drive or dairy development between dairy breeds as well as within breed. From this standpoint FCM/W should appeal to those agencies working with the dairy breeds collectively, as producers of milk.

Equation (d) requires a slight modification of the discussion under equation (c). In equation (c), FCM/W is expressed as a linear function of W and A, while in equation (d), FCM/W is expressed as a curvilinear function of A alone by introducing a term in A^2. The coefficients of A and A^2 both are significant as judged by their standard errors. By equation (d) 1,000 $FCM/W = 37.8$ at $A = 35$, reaches a maximum of 41.6 at $A = 86$, and then declines to 30.6 at $A = 174$. As in the case of W in equation (a), the declining phase is not very reliable and is of little practical importance because of the infrequent occurrence of lactations very far advanced on the descending limb of the curve. The descending limb is of some theoretical interest as indicating senescence in the intensity of lactation metabolism in old age.

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[3] Generally speaking, it is permissible to say that the amount of body protein in cows of different liveweights is proportional to liveweight. But there is a good deal of assumption in saying that the amount of protoplasm involved in lactation is proportional to W as here defined. The assumption is encouraged by the fact that milk-energy yield is proportional to W in the present data, according to the fitted equations (b) and (c). It must be recognized, however, that W is affected by fatness (a body food reserve available for lactation needs) as well as by size in the sense of body dimensions. The problem is complicated. However, the weight of visceral organs need not be a factor of great importance because the visceral organs have a wide margin of safety above the point of being a limiting factor in the amount of lactation.

Equation (d) indicates that the regression of FCM/W on age is curvilinear and significant in the probability sense. Can the increase of 3.8 in 1,000 FCM/W from the youngest age to age of maximum be ignored? In consideration of this question it is of interest to see how age-corrected FCM behaves in relation to age in the same 231 lactations. Applying the official age-correction factors of the Holstein-Friesian Association to each of the 231 lactations, the following within-cow equation emerges:

$$A\text{-}CFCM = 49.76 + (0.260 \pm 0.069)\, A - (0.00154 \pm 0.00040)\, A^2 \qquad \text{(e)}$$

Equation (e) shows that the official age-correction factors do not completely remove age changes in FCM yield for these 231 lactations. According to the equation, age-corrected $FCM = 57.0$ at $A = 35$, increases to 60.8 at $A = 85$, and then decreases to 48.5 at $A = 174$. Comparison of equations (d) and (e) shows that FCM/W is practically as close to being independent of age as is age-corrected FCM for these 231 lactations, using the official age-correction factors.

The derivation of age-correction factors has long been a favorite occupation of investigators engaged with the biometric analysis of milk records. Following are two equations derived from the present 231 lactations:

$$\text{In total,} \quad FCM = 23.26 + 0.7252\, A - 0.0033548\, A^2 \qquad \text{(f)}$$

$$\text{Within cow,} \quad FCM = 24.42 + 0.7296\, A - 0.0036064\, A^2 \qquad \text{(g)}$$

Equation (f), dealing with the 231 lactations in total (the usual method of approach), indicates an age-correction factor of 1.35 for $A = 35$ (birth age 25.5 months). Equation (g), dealing with lactations within cow (theoretically a more refined method of approach), indicates an age-correction factor of 1.40 for $A = 35$. The official factor is 1.25.

Equation (g) appears to be a better way of deriving the age-yield relation generalized for the same cow than is the ratio method of Sanders (5), but the authors have no desire to add to the multiplicity of age-correction factors now in the literature. Consequently, use of lactational drive, measured as 1,000 FCM/W which has no need of age correction (and no need of weight correction within breed or probably within species), is advocated.

SUMMARY AND CONCLUSIONS

This paper deals with 57 registered Holstein cows in the Nebraska Station herd at Lincoln, Nebraska, each cow having three or more lactations, a total of 231 lactations. Age at calving (A), liveweight within 31 days after calving (W), milk-energy yield per day for the 243-day partial lactation (FCM), and lactational drive (1,000 FCM/W) are accurately known for each lactation of each cow. Various equations have been fitted by least squares to the observations, both in total and within cow. The within-cow equations are presumed to give the best insight of the relationships between the variables from a biological point of view.

When *FCM* is expressed as a linear function of *W* and *A*, within cow, the coefficient of *W* is relatively large and highly significant while that of *A* is very small and not significantly different from zero. The constant term is very small. The independent effect on *FCM* of one pound in *W* is equal to the independent effect of 3.44 years in *A*. From this within-cow result it is concluded that for these 231 lactations, dealing with *A*, *W* and *FCM* as they actually exist under natural conditions, it is biologically unsound to correct yield for age because age has no effect on yield independent of live-weight.

When lactational drive (1,000 *FCM*/*W*) is expressed as a linear function of *W* and *A*, the constant term is large and the coefficients of *W* and *A* both are small, each being smaller than its own standard error, or neither one is significantly different from zero. From this within-cow result it is concluded that lactational drive (1,000 *FCM*/*W*, as defined) is a directly comparable measure of dairy development or intensity of lactation metabolism within these 231 lactations, being completely independent of both age and live-weight.

From the present within-cow equations, from previous equations, and from a metabolic or dynamic point of view, the conclusion is reached that lactational drive (as defined) affords a biological common denominator for dairy cattle as a whole, with respect to yield of milk.

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EFFECTS OF HIGH VITAMIN A INTAKE ON MILK AND FAT YIELDS AND ON VITAMIN A CONSTITUENTS IN MILK, BLOOD, AND LIVERS OF DAIRY COWS[1]

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Recognition of the importance of vitamin A constituents in the diets of dairy cattle has resulted in extensive investigation of the effects of various vitamin A supplements on the health of the cow and on the properties of milk. A summary (2) of reports on the effects of feeding crude cod-liver oil and menhaden fish oil to lactating cows indicates that when these oils are given in sufficient quantities to augment the vitamin A potency of the milk, the percentage of fat is reduced. In recent years the feeding of vitamin A supplements such as shark liver oil and vitamin A concentrates has come into common use for certain classes of livestock. The addition of these high potency vitamin A materials to the diets of cows has increased, in varying degrees, the concentrations of this vitamin in the blood (5, 8, 9, 15), in the milk (1, 2, 7, 8, 10, 15, 18, 21), and in the liver (6), but has produced discrepant effects on yields of milk and of fat (1, 2, 7, 8, 10, 15, 18, 21, 23, 24).

The variability and the diversity of the results reported warranted further study of the effects of feeding vitamin A supplements to dairy cows maintained in a good state of nutrition. Hence, in an investigation designed to ascertain the effects of prolonged supplementation of massive amounts of vitamin A on the course of mastitis, additional observations were made on the yields of milk, the percentage of fat, and the concentrations of vitamin A in the milk, the blood, and the liver. The results from this phase of the investigation are reported herein.

EXPERIMENTAL PROCEDURES AND RESULTS

Grouping and Care of Experimental Animals

Experimental cows. Two comparable groups of dairy cows, the control and the supplemented, were used in this trial. The following factors, in the order listed, were considered in grouping the cows: breed, mastitic history, daily milk yields, stages of gestation and lactation, and body weights. Each group at the beginning of the trial consisted of nine mature cows, two Ayrshires, two Guernseys, and five Holsteins. Six of the cows in each group were lactating, being past the stage of peak production but not sufficiently

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advanced in gestation to accentuate the rate of decline in yield; the remaining three cows, two Ayrshires and a Holstein, were in the early stages of the dry rest period, from 44 to 52 days prepartal. These dry cows were included to determine the effects of prepartal supplementation on postpartum changes.

In addition, two non-lactating Ayrshire cows in the last month of gestation were used to study in detail the effects of level of vitamin A intake on the changes in carotenoids and vitamin A of the serum during the terminal stages of gestation and early period of lactation. The two cows had practically the same carotenoid and vitamin A content of the serum before they were subjected to experimental conditions.

Feeding and management. Prior to the initiation of the trial and throughout the experimental period of 12 weeks, during the months of November, December, and January, all cows of the two groups received a standard milking herd ration consisting of a 16 per cent protein concentrate mixture, Atlas sorgo silage, and alfalfa hay. The carotenoid content of the hay, on the moisture-free basis, ranged from 0.06 mg./g. in the early part of the trial to 0.03 mg./g. in the latter. The lactating cows were fed daily 1 lb. of concentrate mixture for each 4 lbs. of 4 per cent fat-corrected milk, 20 to 25 lbs. of silage per 1,000 lbs. body weight, and hay *ad lib.* The dry cows were fed daily 8 lbs. of the concentrate mixture per 1,000 lbs. of body weight and the roughages, the same as for the lactating cows. In addition to the barn feeds, the cows of the two major groups grazed on rye pasture 30 to 40 days before initiation of the experiment and 16 days following.

Throughout the experimental period, all cows (both the dry and the lactating) in the supplemented group received daily 1,250,000 USP units of vitamin A in a powdered medium.[2] Since this vehicle, described as a "soybean oil meal like" product, supplied nutrients in addition to vitamin A, the cows of the control group received soybean oil meal in quantities equal to the amount of vitamin A supplement fed to the other group. These additional feeds and supplements were given once daily in combination with the concentrate mixture. Considerable quantities of the vitamin A supplemented mixture were refused during the first several days of feeding, but after the cows became accustomed to the foreign flavor, no consumption difficulties were encountered.

All cows were subjected to standard herd management, which included feeding and milking twice daily, exercise whenever weather conditions permitted, and free access to water, common salt and hay in the same paddock.

Yields of Milk and Concentrations of Fat and of Vitamin A Constituents

Milk yields and fat percentages. Detailed records of the milk yields of individual cows were made throughout the experimental period. Samples of milk were collected during two consecutive milkings each week for the determination of fat concentration by means of the standard Babcock procedure. The weekly milk and fat yields of the respective groups were summarized and subsequently converted to a 4 per cent fat-corrected basis according to the Gaines (11) formula. The yields of milk and fat (fig. 1) revealed no marked differences that could be ascribed to the rations fed.

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[2] "Dry vitamin A", having 2,500–2,700 USP units per gram.

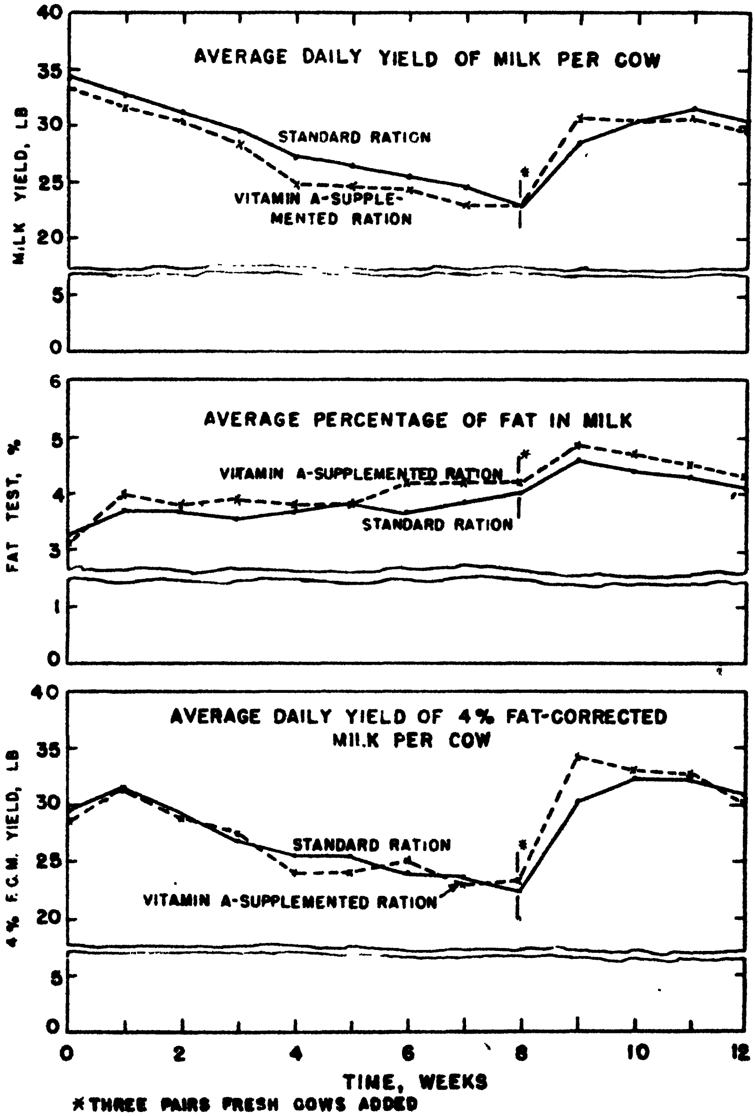

FIG. 1. The effects of dietary supplementation of vitamin A on milk yields and on fat percentages.

Initiation of vitamin A supplementation during the early stages of the dry rest period revealed no advantage in production during the first month of lactation. Thus it appears that the daily addition of massive amounts of "dry vitamin A" to the dairy ration used in this experiment neither augmented total milk yield nor suppressed the fat percentage of the milk.

Concentration of carotenoids and vitamin A in the milk fat. During the terminal week of the experiment, a 1-day composite sample of milk was collected from each of seven individual cows in the respective groups for assays of carotenoids and vitamin A. (Milk from two pairs of cows was excluded because of mastitis complications.)

The analytical method employed was a modification of the double extraction procedure of Boyer *et al.* (4). A total of 70 ml. of ether was used in extracting vitamin A and carotenoids, and the volumes of wash solutions were adjusted accordingly. The washed solution of extracted vitamin A and carotenoids was dried by means of anhydrous sodium sulphate, and the ether was removed from the extract by suction while heating in a water bath at 50–60° C. The residue was dissolved in 15 ml. of Skellysolve B. A 10-ml. aliquot was used for the final determination of carotenoids and of vitamin A. Shaking the ether solution with 5 ml. of a saturated solution of sodium chloride, as outlined in the original method, was omitted. Photometric measurements were made on a Coleman spectrophotometer, model 11, modified to reduce light intensity. Since the fat percentage in the samples was variable, the carotenoid and the vitamin A values were expressed as concentration per unit of milk fat.

TABLE 1

Average concentrations of vitamin A and carotenoids in the milk fat of cows on different levels of vitamin A intake

Daily supplement of vitamin A	Breed of cows	No. of cows	Vit. A and carotenoids of milk fat	
			Vitamin A	Carotenoids
			γ/g.	γ/g.
None	Guernsey	2	6.4	5.8
	Holstein	3	7.3	3.4
	Ayrshire	2	7.8	4.1
1,250,000 USP units	Guernsey	2	24.1	2.5
	Holstein	3	30.5	4.1
	Ayrshire	2	36.1	1.9

The average, by breeds, of vitamin A in the milk fat from supplemented cows was approximately four times higher than that from the control cows. The carotenoids throughout were low, but, in accord with other reports (2, 8, 9, 10, 15), tended to be lower in the milk fat from vitamin A supplemented cows. There was, however, an exception in the case of two of the three pairs of cows of the Holstein breed; hence the average for this breed did not reveal the reduction (table 1). Since preliminary assays were not made, individual differences could account for these exceptions.

Concentration of Carotenoids and Vitamin A in Blood Serum

Total carotenoids and vitamin A were measured in the serum of venous blood. Since the cows were on a high carotenoid intake during the early stages of the experiment, the non-saponification method of Boyer *et al.* (3) was chosen in lieu of the more generally used Kimble (16) procedure, which is recognized to be inaccurate for vitamin A measurements in the presence of high concentrations of carotenoids (15). Though the non-saponification method of Boyer *et al.* (3) apparently is unsuitable for dog blood, which is presumed to be high in the ester form of vitamin A, this procedure was reported to be applicable to normal bovine blood.

TABLE 2

Average concentrations of vitamin A and carotenoids in the blood serum of cows on different levels of vitamin A intake

Daily supplement of vitamin A	Breed of cows	No. of cows	Vitamin A and carotenoids in serum*			
			Dec. 11–26, 1944		Jan. 22–26, 1945	
			Vitamin A	Carotenoids	Vitamin A	Carotenoids
			γ/100ml.	γ/100ml.	γ/100ml.	γ/100ml.
None	Guernsey	2	23.2	1007	22.8	487
	Holstein	5	20.2	617	18.6	385
	Ayrshire	2	8.7†	279†	21.6	340
1,250,000 USP units	Guernsey	2	23.5	501	27.1	239
	Holstein	5	26.0	310	24.6	177
	Ayrshire	2	23.6†	234†	33.9	245

* Boyer *et al.* (3) non-saponification procedure.
† One week postpartum.

Effect of the diet on the concentration of vitamin A and carotenoids in the blood serum. The carotenoid and the vitamin A values in table 2 are averages of assays of blood serum samples collected from individual cows of the three breeds in the respective groups. The first period of collection, a span of 14 days, was 6 weeks after the initiation of the trial and approximately 1 month after discontinuing rye pasture; the second period of 7 days was 1 month later, near the termination of the trial.

The vitamin A content of the blood serum of the supplemented cows was higher than in the controls, but the carotenoid values were lower. The magnitude of the difference in vitamin A concentration tended to vary with breeds, being least in the Guernsey and greatest in the Ayrshire.

The concentration of carotenoids in the serum from the Guernsey and the Holstein breeds decreased from the first period to the second, but the vitamin A values showed no significant changes. This marked decline of the carotenoids probably was due to the continued reduction of reserves following removal from rye pasture and to a decrease in the carotene content of the hay consumed. Though the Ayrshires were subjected to the same dietary regime as the other two breeds, the vitamin A was low during the first period as a result of a reduction associated with parturition (5, 17, 22). With postpartum physiological readjustments, the vitamin A concentration increased to a decidedly higher level, whereas the carotenoids changed very little.

Relation of the analytical procedure to vitamin A values of serum. Since the differences in vitamin A concentration in the serum from cows of the respective groups were not of the magnitude observed in similar experiments by other investigators (9, 15), the non-saponification procedure of Boyer *et al.* (3), by which the values in table 2 were obtained, was compared with the Kimble (16) method. The comparison was made near the termination of the trial when the carotenoid content of the blood serum was sufficiently low to minimize interference.

TABLE 3

Comparison of methods of determining vitamin A in the serum of cows on different levels of vitamin A intake

Daily supplement of vitamin A	Breed of cows	No. of cows	Vitamin A values by different methods		
			Kimble	Boyer *et al.**	Difference
			γ/100ml.	γ/100ml.	γ/100ml.
None	Guernsey	2	22.6	21.5	1.1
	Holstein	1	20.7	18.7	2.0
	Ayrshire	2	20.5	18.1	2.4
	Av.	5	21.4	19.6	1.8
1,250,000 USP units	Guernsey	2	29.7	23.7	6.0
	Holstein	1	39.6	30.5	9.1
	Ayrshire	2	45.1	32.9	12.2
	Av.	5	37.8	28.8	9.0

* Non-saponification method.

The Kimble (16) procedure yielded higher values throughout than did the non-saponification method of Boyer *et al.* (3), but the average difference was greater in the vitamin A supplemented group, 31.3 per cent, than in the non-supplemented group, 9.2 per cent (table 3). Further comparisons of the results revealed that the average values for the supplemented cows were 76.6 per cent higher by Kimble but only 46.9 per cent higher by Boyer *et al.* The lower values by the non-saponification method of Boyer *et al.*, particularly in serum from cows receiving dietary vitamin A, suggested that either this procedure failed to include all the vitamin A or the Kimble method yielded excessively high values. Recent observations (19) indicate that as the vitamin A content of serum increases from vitamin A feeding, the values obtained by the non-saponification procedure of Boyer *et al.* tend to be too low. In view of this, it is probable that the total vitamin A in the serum of the cows receiving the vitamin A supplemented ration was nearer the level indicated by the Kimble method than that by the Boyer *et al.* This phase of the problem is being investigated further.

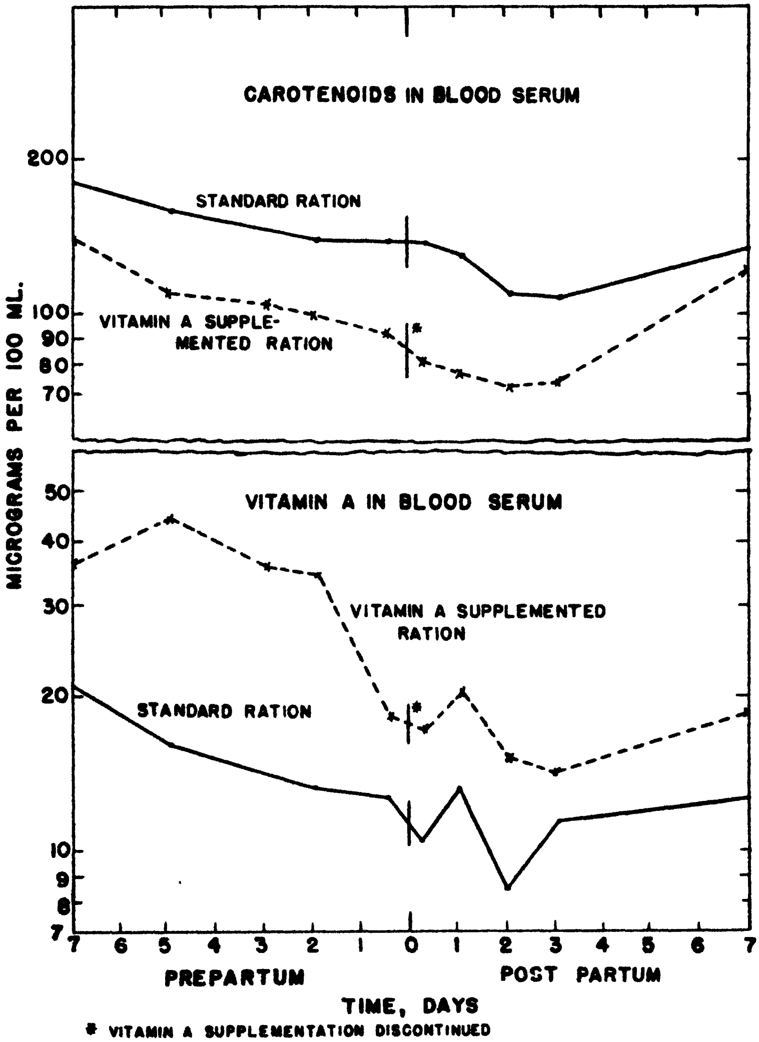

FIG. 2. The effect of prepartal dietary supplementation of vitamin A on the carotenoid and the vitamin A concentration in the blood serum of the dairy cow during the stages of terminal gestation and initial lactation.

A preferable method would have been the saponification procedure outlined by Boyer *et al.* (3), but early attempts to apply it yielded spurious results. Subsequent investigation revealed that the difficulty was due to a contaminant, presumably aldehyde (12), in the alcohol used.

Effect of prepartal vitamin A intake on the changes of carotenoids and

vitamin A in the serum of the parturient cow. As suggested by the average vitamin A values in the serum from cows of the Ayrshire breed (table 2), the vitamin A supplementation did not prevent the usual gestational reduction but did maintain a higher level than observed in the control cows. This is illustrated further (fig. 2) by the prepartum and the postpartum changes in the blood serum of an Ayrshire cow from each of the two dietary groups, control and vitamin A supplemented. Though the two cows selected had about the same carotenoid and vitamin A levels prior to supplementation. dietary vitamin A increased the vitamin A of the blood serum but reduced the carotenoids, as measured by the Kimble method (16) of analysis. The curves, on a semi-logarithmic scale, show that in both animals the prepartum rate of vitamin A decline was more pronounced than that of the carotenoids. The drop of vitamin A, however, was more precipitous in the serum of the cow receiving vitamin A. A temporary rise in the concentration of vitamin A was noted in both cows the day following parturition. Further data (19) indicate that this phenomenon also is common in other cows, but the frequency of occurrence is unknown and the factors involved are obscure. The minimum postpartum concentration usually occurred about the third day unless complicated by infections (5). Whether or not continued postpartum supplementation would have accelerated the rate of recovery when adequate liver stores were available is problematical.

Concentration of Vitamin A in the Livers and in the Serum of Cows Slaughtered

Livers were salvaged from seven cows to determine the effect of vitamin A intake on storage. During the week prior to slaughtering, blood samples were collected for vitamin A determinations. The assay procedure for livers was a slight modification (25) of the Guilbert and Hart (13) method. and for blood serum the non-saponification procedure of Boyer *et al.* (3) was used. With the exception of one animal, no. 169, vitamin A supplementation was continued to within 24 hours of slaughtering.

When an abundance of vitamin A was present in the daily ration, the cows accumulated pronounced liver reserves of this vitamin, approximately four times the amount detected in the livers of cows on unfortified rations (table 4). Though several of the livers had isolated abscesses, this pathological condition apparently did not interfere with vitamin A storage. If it is assumed that prior to cessation of supplementation the liver reserves of cow no. 169 had reached the same general level as in the other cows of her group, the rate of depletion of vitamin A stores was rapid. The vitamin A concentration in the serum from the individual cows revealed, in accord with the report of Braun (6), no correlation between the liver reserves and the levels in the blood serum.

TABLE 4

Concentrations of vitamin A in the livers and in the serum of cows on different levels of vitamin A intake

Daily supplement of vitamin A	Breed of cows	Herd no.	Wt. of liver	Vitamin A		Remarks
				Liver	Serum	
			kg.	$\gamma/g.$*	$\gamma/100ml.$	
None	Holstein	104A	8.2	150	26.4	Non-breeder. Healthy liver.
	Ayrshire	222A	7.5	142	23.4	Brucellosis reactor. Healthy liver.
	Jersey	332A	5.2	138	19.3	Brucellosis reactor. Healthy liver.
1,250,000 USP units	Holstein	144	9.5	600	28.8	Non-breeder. Mastitic. Abscesses in liver.
	Holstein	161	8.6	733	23.8	Mastitic. Healthy liver.
	Holstein	169	8.9	421	27.2	Vitamin A withheld 20 days preceding slaughter. Mastitic. Abscesses in liver.
	Holstein	173	9.8	867	23.2	Mastitic. Malignant growths in carcass.

* Wet basis.

DISCUSSION

The data presented on milk and milk fat confirm reports by other investigators (2, 10, 15, 18, 21) indicating that production is not stimulated by vitamin A supplementation when the lactating cows are in a good state of nutrition. Probably when favorable responses are elicited by vitamin A feeding (1, 7, 8, 23, 24) either this vitamin *per se* or some other nutrient for which an increased level of vitamin A tends to compensate is the limiting factor. The apparent adequacy of rations for lactating cows at any particular time may be misleading unless cognizance is taken of their nutritional history, productive capacity, and feed consumption. Wilson (24) suggested that access to good quality roughages high in carotene does not insure adequate intake, particularly by high producing cows that have much of their feed capacity utilized by rations low in vitamin A active substances. It is conceivable that in many herds a slight submarginal deficiency of vitamin A may prevail as a result of unrecognized depletion. In these cases a favorable production response to vitamin A feeding would be expected.

Since "dry vitamin A" supplementation did not depress the milk fat percentage, as is observed commonly when cod-liver oil is fed, it is probable that the unsaturated fatty acids that are believed to cause the toxic reaction were not present in sufficient amounts to affect the mammary function. This indicates that the vitamin A concentrate used in this investigation may be fed in sufficient quantities to increase the vitamin A potency of the milk without adversely affecting the fat content.

The observed increases in the vitamin A potency of the milk from dietary supplementation are in accord with the findings of others (1, 2, 7, 8, 9, 10, 15, 18, 21, 24). This means of fortifying milk, however, is uneconomical since the efficiency of secretion of ingested vitamin A is exceptionally low (8, 9, 15). Moreover, the concomitant reduction of carotene with increases in vitamin A (2, 8, 9, 10, 15) suggests that dietary vitamin A possibly reduces the nutritional value of carotenoids in the ration, thus presenting a provocative problem.

The interference of carotenoid metabolism has been ascribed to vitamin A *per se* rather than to other associated constituents (9, 20). Several possible explanations of this phenomenon have been presented. According to Hickman (14), " *in vitro* experiments show that vitamin A is a specific pro-oxidant for beta-carotene, lycopene, and probably zeaxanthin." Data supplementary to those already presented showed that the carotenoid concentration in a composite sample of feces from cows on a standard ration was approximately the same as in a similar sample from vitamin A supplemented cows. The vitamin A content of the feces from the latter group, however, was about 60 per cent higher. Either vitamin A was not a factor affecting the carotenoids in the bowel or it simultaneously suppressed absorption and accelerated oxidation. Recent studies (20) with chickens revealed retarded pigmentation of the shanks after cessation of vitamin A supplementation. Deuel *et al.* (9) noted a similar post-supplementation lag in recovery of carotenoid levels in dairy cows. The foregoing observations indicate that the carotenoid suppression is not exclusively an intestinal phenomenon.

A further explanation advanced by Deuel *et al.* (9) is that increases of vitamin A accelerate the destruction of carotenoids in the tissues through the development of a new enzyme system. It was suggested also that this enzyme system may destroy vitamin A. If this proves to be correct, feeding massive amounts of vitamin A over a prolonged period may be detrimental to the organism instead of beneficial.

Another viewpoint is that vitamin A may aid in the conversion of certain carotenoids to this vitamin, thus enhancing the accumulation of a maximum reserve. If it is assumed further that the capacity for storage in the body is limited, the suggested reduction of vitamin A (20) might be an accelerated elimination after the threshold is reached instead of a process of systemic destruction.

Though a decline of carotenoids and vitamin A of the blood seems to be a normal accompaniment of parturition (17, 22), the specific causes of this depression are obscure. A drop occurs regardless of the prepartal intake, but Kuhlman and Gallup (17) observed that the percentage decrease of carotene was related directly to its level in the plasma. This, as indicated by data reported herein, seems to apply to vitamin A levels also.

Attempts to associate these changes of carotenoid and vitamin A concentrations of the blood with mammary function have yielded negative results. Although the secretion of colostrum withdraws vast amounts of nutrients from the blood, Sutton *et al.* (22) found no statistical correlation between levels of carotene and vitamin A of plasma and the output of these constituents in colostrum. Braun (5) reported that a temporary reduction of vitamin A occurred when cows aborted, under which conditions colostrum secretion would be negligible. Similar reductions of carotenoids and vitamin A were observed in a mammectomized cow following premature calving (26). As suggested by Sutton *et al.* (22), many factors and complex interrelationships may be involved. Investigation of the endocrinological aspects of the problems may aid in clarification.

The regulatory role of the liver in maintaining vitamin A concentrations in the blood, particularly in advanced gestation and early lactation, has not been elucidated. The reserves in this organ apparently are not a limiting factor except at subnormal storage levels. The changes in the vitamin A concentrations of the liver during this critical transitory period in the reproductive cycle merit study.

The amount of vitamin A in the livers of cows can be modified, as noted by others (6, 13), by dietary means. Data presented by Braun (6) suggested an optimum level for storage in this organ, but a comparison of his results with those reported herein indicates a wide margin between the optimum and the possible maximum levels attainable. Though the concentrations in the livers of the supplemented cows were at a uniformly high level, this does not indicate that the maximum was attained. Present information on the subject raises the question of whether or not the maximum attainable concentration of vitamin A in the liver is the same from carotenoid feeding as from vitamin A supplementation.

Most nutritional studies with dairy cattle have been directed toward determinations of effects of deficiencies and the establishment of minimum requirements. The results of this study suggest the need for considering the results from optimum and/or excess quantities of nutrients in the diet.

SUMMARY

The effects of daily supplementary feeding of 1,250,000 USP units of vitamin A in the form of a dry concentrate ("dry vitamin A") to individual lactating cows over a period of 3 months were compared with the results from similar cows on a standard dietary regime. The following conclusions were reached:

1. Vitamin A feeding had no significant effect on total milk and fat production.
2. The "dry vitamin A" concentrate did not depress the milk fat percentage.

3. The high intake of vitamin A increased the concentration of this vitamin markedly in the milk fat but tended to suppress the carotenoid content.

4. Prolonged dietary supplementation of vitamin A increased the level of this vitamin in the blood serum but reduced the carotenoid values. The apparent magnitude of the vitamin A values varied with the analytical procedure used in the assay.

5. Supplemental feeding of vitamin A throughout the terminal stages of gestation did not prevent the characteristic declines at parturition but did maintain a higher level at this period than observed in non-supplemented cows.

6. The differences in vitamin A intake were reflected in the concentrations of vitamin A in the liver. There was no evidence of a correlation between vitamin A levels in the blood and in the liver of any of the cows that were slaughtered.

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COBALT IN COWS' MILK[1]

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In continuation of a comprehensive project on the minerals of cows' milk, three reports on which have been published (1, 2, 3), the effect of feeding cobaltous acetate on the cobalt content of milk has been investigated. The feeding trials were conducted during the winter of 1943–44, but because of difficulty in finding a sufficiently sensitive method for the determination of extremely minute amounts of cobalt, the analytical work has been completed only recently.

Comparatively little information is recorded regarding the cobalt content of milk. Probably because of the difficulties of analysis, some of the earlier investigators (5, 7, 8, 13, 14) either do not report cobalt as a constituent of milk or else claim it to be absent, although one pair of workers (7) notes that "the complete absence of cobalt is somewhat unexpected, for it is an element with active biological properties." In 1933, Stare and Elvehjem (10) concluded that the cobalt content of milk is less than 0.01 mg. per 100 g. (100 μg. per liter of milk). By 1938, methods had been sufficiently refined so that Underwood and Elvehjem (12) report a range of 8–18 μg. per liter with an average of 11 μg. The most recent value noted is that given by Ellis and Thompson (9) in 1945, who report 0.64 μg. per liter. Other values recorded range from 1 μg. per liter (11) to 10–15 μg. per liter (5).

Data relative to the influence of the amount of cobalt in the feed on the cobalt content of milk are limited. In 1942, Askew (4) reported that cows having access to cobaltized salt licks produced milk containing 0.020–0.022 p.p.m. (20–22 μg./l.) of cobalt, as compared to 0.010–0.015 p.p.m. (10–15 μg./l.) in the milk of control animals. In the light of recent refinements of method, these values are probably too high, but the relative relationship is of interest. Comar *et al.* (6) noted a very small, unspecified amount of cobalt in milk when the radioactive element was introduced into the blood stream of cows. However, when it was introduced directly into the rumen, cobalt was not detected in the milk.

EXPERIMENTAL

The procedure was similar to that described in an earlier paper (1). Eight cows were divided into two groups of four each, each group consisting of an Ayrshire, a Guernsey, a Holstein, and a milking Shorthorn. Three of the breed pairs were matched with respect to stage of lactation, none being beyond the 12th week in lactation when the trial was started. The fourth

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pair (the Shorthorns) were in the 8th and 18th week of lactation, respectively, at the start, a matched pair not being available. One group received the supplement during December and January; the other group received it during February and March. The amount of cobaltous acetate fed was 500 mg. daily or the approximate equivalent of 120 mg. of elemental cobalt. Except for the feeding of the supplement, the rations and management of the two groups were identical.

Composite 2-day milk samples of two liters each were taken from each cow once a month. Cobalt was determined in triplicate on 500-ml. portions of each sample by the method of Ellis and Thompson (9), using the alternate carbamate extraction procedure. This method, although requiring much preliminary procedure in the purification of reagents, extreme precautions in technique and the scrupulous cleaning of glassware in order to avoid contamination, proved to be very satisfactory. The standard curve for cobalt established at the start was checked twice throughout the course of the work and was found to give good agreement; recovery of added amounts of cobalt ranged from 97 to 104 per cent of theory. A Model 11 Coleman spectrophotometer was used at a wave length of 345 millimicrons with filter PC6 and absorption tubes 50 mm. long and 10 mm. in diameter, with a capacity of approximately 3 ml. Final volume of the unknown solution was usually between 4 and 5 ml. A continuous glass still for production of double distilled water is a prerequisite for this type of work.

Previous to adoption of this method an older method which makes use of nitroso-R salt as the specific reagent for cobalt was given extensive trial, but proved quite unsuited to the purpose. Spectrographic analysis also was tried but was abandoned when it failed to reveal the presence of cobalt in dilutions of the magnitude anticipated. At already noted, other investigators (5, 13) also have been unable to identify cobalt in milk ash by means of the spectrograph.

RESULTS

The values obtained are summarized in table 1. The amount of cobalt occurring naturally in these milks averaged about 0.6 μg. per liter, with a range from 0.2 to 1.14 μg. These values are of the same order of magnitude as those reported by Ellis and Thompson (9) and Sylvester and Lampitt (11). There was some tendency for the amount of cobalt in the control milks to diminish as the season advanced. A similar tendency was noted in earlier work on manganese (1). The question raised at that time regarding that element appropriately may be raised with respect to cobalt, *viz.*: Does this mean that possibly, during the pasture season, cows store a reserve of the element which tends to become depleted as the winter season on dry feed proceeds?

Without exception, the milk from a cow receiving supplemental cobalt was higher in the element than the milk from her breed mate not receiving

TABLE 1

Effect on cobalt content of the milk of feeding cows cobaltous acetate
(Micrograms of cobalt per liter of milk)

Month	Cows on control ration					Cows receiving supplemental cobalt				
	1st half of the season									
	*A327	G658	H581	S62	Average of all four	A298	G640	H567	S38	Average of all four
December	1.0	1.0	0.6	0.9	0.9	2.3	1.6	1.0	1.5	1.6
January	0.8	0.7	0.5	0.3	0.6	2.6	2.9	1.4	1.8	2.2
Av. 1st half	0.9	0.9	0.6	0.6	0.7	2.5	2.3	1.2	1.6	1.9
	2nd half of the season									
	A298	G640	H567	S38	Average of all four	A327	G658	H581	S62	Average of all four
February	0.9	1.1	0.5	0.4	0.7	2.5	2.3	1.4	5.9†	3.0
March	0.2	0.2	0.2	0.2	0.2	2.7	2.9	0.9	5.0†	2.9
Av. 2nd half	0.6	0.7	0.4	0.3	0.5	2.6	2.6	1.2	5.4	2.9
Av. entire season	0.7	0.8	0.5	0.4	0 6	2.6	2.4	1.2	3.5	2.4

* The initial letter prefixed to each cow's number indicates the breed.

† Although there is nothing in the history of these two samples that would lead one to suspect contamination, they are so much out of line with the other values obtained that their validity may be open to question. However, since a high value for this cow was obtained in two successive months, the results are included in the average.

it. The increase ranged from less than two-fold (1.7—Holsteins—December) to twenty-five-fold (Shorthorns—March). As noted in the table, the latter value may be questioned. Of more interest is the average increase which was four-fold, a value which is highly significant statistically.

Although some variations between breeds are evident, the only consistent differences are the relatively low values for cobalt in Holstein milk, both when the cows were on the control ration and when they were receiving the supplement.

The obvious possible significance of these results lies in their application to calf nutrition. In our experience, young stock have shown greater susceptibility to the nutritional anemia which is characteristic of cobalt deficiency than have older cattle. In the light of these results it would seem that in areas where cobalt deficiency is common, the requirements of calves for this element might most naturally and logically be supplied through the milk of cows whose rations have been fortified with supplemental cobalt. Since many of our feed manufacturers now include cobalt regularly in the formulation of ready mixed rations for dairy cows, the use of such rations would tend to automatically supply the needs of the young calf for cobalt. Under ordinary circumstances the calf's requirements for cobalt may not be met since some of the control milks in this investigation contained less cobalt than was found in the drinking water supplied to the cows (0.21 μg./l. as compared with 0.26 μg./l.).

SUMMARY

Cobaltous acetate was fed as a supplement (500 mg. daily) to the rations of eight cows for a period of 2 months by the double reversal method and the milk was analyzed for cobalt. The results revealed that feeding the supplement consistently raised the amount of cobalt in the milk. The average increase was four-fold. The milk from cows receiving the supplement averaged 2.4 μg. of cobalt per liter in contrast with 0.6 μg. per liter when the cows were on the control ration. The possible significance of the results is discussed briefly.

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THE NUTRITIVE VALUE OF FRACTIONS OF BUTTERFAT PREPARED BY COLD CRYSTALLIZATION[1]

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In past work the following methods have been employed to concentrate and characterize the factor(s) in butterfat responsible for its superior nutritive value as compared with corn oil: lead soap separation and steam distillation of butterfat fatty acids (2, 10), fractional distillation of the methyl esters of butterfat fatty acids (5), and chromatographic procedures (3). Since many of these require conditions conducive to chemical changes, cold crystallization of an acetone solution of butterfat was selected as a procedure most likely to yield fractions which had undergone little chemical transformation.

Henry *et al.* (7) reported the nutritive value of three fractions of butterfat prepared by cold crystallization from an acetone solution of 0° C. Two of these products with iodine numbers of 19 and 47 were fed to rats in a skimmed milk ration. The rate of growth was best on the diet containing the liquid fraction, but the authors stated that the small differences in gains between groups were without statistical significance. In 1946 Jack *et al.* (8) published the results on the nutritive value of five different fractions of butterfat prepared by low-temperature crystallization from hexane. Successive temperatures of – 7, – 13, – 23, and – 53° C. were used, and in each case the precipitate was removed. The final filtrate was concentrated and yielded the fifth fraction. To test the effect of solvent treatment on the nutritive value of the fat, butterfat was dissolved in hexane and the solvent removed by distillation. The fractions as well as the treated and untreated butterfat were incorporated into a synthetic type ration and fed to groups of rats. The poorest gain was made by the animals receiving the – 7° C. precipitate (m.p. 53° C.) and the best gain was made by the animals fed either the untreated butterfat or the – 53° C. filtrate rations. The solvent-treated butterfat proved to be definitely inferior to the untreated fat.

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[2] Government of India Research Fellow.

The experiments described in the present paper concern the application of cold crystallization to samples of butterfat obtained at various times throughout the year. The nutritive value and some chemical and physical constants of the fractions prepared are reported.

EXPERIMENTAL

The butterfat used in these experiments was prepared by decantation and filtration of melted (65° C.) unsalted sweet cream butter obtained from the University Creamery. The acetone was redistilled in an all glass still from calcium chloride before being used. One kilogram of melted butterfat was dissolved in 10 liters of acetone, and after being thoroughly stirred, the solution was cooled to $-4°$ C. and kept at this temperature for 24 hours. Filtration of the yellow supernatant liquid from the white granular precipitate was accomplished by means of a submerged pressure filter. The precipitate was recrystallized twice from 5 liters of acetone at $-4°$ C. The filtrates were combined and concentrated under partial vacuum, using a nitrogen ebullition tube. The temperature was kept below 20° C. except during the removal of final traces of solvent, when more heat was applied and a vigorous stream of nitrogen was used. The residue was reinforced with the proper amounts of the fat-soluble vitamins and then was stored at $-6°$ C. This preparation was designated as butterfat fraction 2 (BF-2). The solid fraction remaining after the recrystallizations was freed of solvent and was vitaminized and stored at $-6°$ C. This product was designated as butterfat fraction 1 (BF-1). The butterfat used in Expt. 1 was obtained in September, 1945; the fractions made at that time were stored for a number of weeks before use. Melting points were determined by a modified Wiley method and iodine numbers by the Hanus method. To eliminate the possible effect that the acetone treatment might have on the nutritive value of the fat fractions, a sample of butterfat was dissolved in acetone and after several hours the solvent was removed as described above.

Corn oil[3] was separated into two fractions in the following manner: 1 kg. of corn oil was dissolved in 5 liters of Skellysolve A and the mixture was cooled to $-45°$ C. by use of an acetone–dry ice bath. After 5 hours at this temperature filtration was accomplished by means of the submerged filter. Because of the "waxy" nature of the precipitate some difficulty was encountered at this stage. Two recrystallizations of the solid material were made from 2,500-ml. portions of solvent at $-45°$ C. The filtrates were combined, concentrated and vitaminized as previously described, and were designated corn oil fraction 2 (CO-2). Similar treatment of the solid product yielded corn oil fraction 1 (CO-1). A sample of corn oil was dissolved in the solvent and then freed of it after several hours to give a solvent-treated corn oil (CO-S).

[3] Mazola brand. Corn Products Refining Co.

Groups of six male weanling rats of the Sprague-Dawley strain weighing 40–45 g. were placed on rations containing each of the following fats: butterfat (BF), corn oil (CO), solvent-treated butterfat (BF–S), solvent-treated corn oil (CO–S), and the fractions BF–1, BF–2, CO–1, or CO–2. The basal ration consisted of:

Casein (alcohol extracted)[4]	20%
Fat	28%
Sucrose	48%
Salts IV (7)	4%
Vitamins per 100 g. of ration:	
Thiamine	200 gamma
Riboflavin	300 gamma
Pyridoxine	300 gamma
Ca pantothenate	1500 gamma
Choline hydrochloride	100 mg.
β-Carotene[5]	560 gamma
α-Tocopherol	2240 gamma
Calciferol[6]	14 gamma
2-methyl-1,4-naphthoquinone	210 gamma

Food consumption was unrestricted. Weekly weight records were kept over a 6-week period, and the results are given in table 1. Because of a marked difference in the appearance of the feces of the rats fed the two fat fractions, feces were collected from each group and analyzed for total fat, ash, and bound fatty acids. The latter determination was carried out by warming the ether-extracted feces with dilute HCl (1:4) for 30 minutes and extracting again with ether. The following results were obtained:

Group	Description of feces			
	Color	% Ash	% Neutral fat	% Bound fatty acids
BF–1	White	15.8	7.8	66.7
BF–2	Black	24.7	15.5	32.4
BF	Mixed	17.8	11.0	50.8

Because of the rapid rate of growth of the animals fed the BF–2 ration, studies were undertaken to investigate this phenomenon further. Fractions similar to those used in Expt. 1 were prepared using butterfat made from butter of December, 1945, and from February, June, July, and September of 1946. In all cases the butter was obtained directly after churning, and the fractionations and animal feeding trials were made without delay. In addition, butter obtained from the Quartermaster Corps of the Army and rated by them as June, 1945, also was tested. In a few instances additional

[4] Extracted for three 2-hour periods with boiling alcohol.

[5] 90 per cent β-carotene and 10 per cent α-carotene.

[6] Crystalline irradiated ergosterol.

fractions were prepared. The nutritional value of these fats and their fractions was tested by incorporating them in the basal diet used in Expt. 1 and feeding each ration to a group of six male weanling rats. The results are given in table 1. Feces collections and food consumptions were made in Expts. 3, 4, and 7, and analyses were made for neutral fat and combined

TABLE 1

Results of cold crystallization experiments

(Each figure represents the average of six rats)

Expt. no.	Group	Description of fat portion	m.p. °C.	Iodine no. (Hanus)	Av. gain	
					4 wks.	6 wks.
					(*g.*)	(*g.*)
1	BF*	Sept., 1945	34.0	33	101	158
Sept., 1945	BF-S	Acetone treated	34.0	33	97	146
	BF-1	– 4° C. ppt.	42.1	27	50	86
	BF-2	– 4° C. filtrate	8.0	47	139	204
	CO*	Mazola		114	88	121
	CO-S	Skellysolve A treated		114	86	125
	CO-1	– 45° C. ppt.		109	88	122
	CO-2	– 45° C. filtrate		121	85	116
2	BF	Dec., 1945			112	163
Dec., 1945	BF-1	– 4° C. ppt.	42.5		90	
	BF-2	– 4° C. filtrate	7.6		117	171
3	BF	Feb., 1946	34.1		129	
Feb., 1946	BF-S	Acetone treated	34.0		123	
	BF-1	– 4° C. ppt.	41.9		116	
	BF-2	– 4° C. filtrate	7.5		124	
	BF-1a	7° C. ppt.	47.1	11	67	
	BF-2a	– 4° C. ppt. minus fraction BF-1a	28.0	17	108	
	BF-3a	Combined filtrates from 1a and 2a	7.2	49	113	
4	BF-M	March, 1946			126	
June, 1946	BF-J	June, 1946	33.0		120	
	BF-1-J	– 5.5° C. ppt.	41.8		109	
	BF-2-J	– 5.5° C. filtrate	9.9		129	
5	BF	July, 1946			128	189
July, 1946	BF-1	– 5.5° C. ppt.			104	153
	BF-2	– 5.5° C. filtrate			125	186
	BF-3	– 5.5° C. filtrate minus the recrystallization filtrates from BF-1			127	188
6	BF	Sept., 1946			101	173
Sept., 1946	BF-1	– 5.5° C. ppt.			111	168
	BF-2	– 5.5° C. filtrate			130	197
7	BF-J	June, 1945, Quartermaster Corps	...	...	115	172
June, 1945	BF-1-J	– 5.5° C. ppt.	42.8	...	75	
	BF-2-J	– 5.5° C. filtrate	12.4	...	125	187
	BF-M	March, 1946	......	......	119	181

* BF = butterfat; CO = corn oil.

TABLE 2

Fecal excretion data for animals fed fats and fat fractions

(All data are based on 3-day pooled samples from six-rat groups, unless otherwise indicated)

Expt. no.	Group	Description of fat portion	Food ingested	Fat intake	Neutral fat excreted	Bound[*] fatty acids excreted	Lipid excreted	Fat absorbed
			(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(%)
3	BF	Feb., 1946	246	68.8	0.91	5.95	6.86	91.2
	BF-1	Acetone treated	228	64.0	1.01	5.23	6.24	91.8
Feb., 1946	BF-1	-4° C. ppt.	204	56.9	1.04	7.64	8.68	84.8
	BF-2	-4° C. filtrate	213	59.6	0.63	3.54	4.16	93.0
	BF-1a	7° C. ppt.	167	46.7	4.01	6.62	10.60	77.3
	BF-2a	-4° C. ppt. minus the 7° ppt.	176	49.2	1.03	6.09	7.11	85.8
	BF-3a	Combined filtrates	203	56.9	0.66	3.69	4.35	92.3
4[†]	BF-J	June, 1946	321	90.0	1.76	8.64	1.04	88.5
	BF-J-1	-5.5° C. ppt.	329	92.0	0.94	3.86	4.81	94.8
June, 1946	BF-J-2	-5.5° C. filtrate	221	62.0	0.43	3.86	4.28	93.1
	BF-M	March, 1946	342	95.9	0.97	3.56	4.53	95.3
7	BF-Q[‡]	June, 1945	152	42.6	1.11	3.30	4.41	89.6
	BF-Q-1	-4° C. ppt.	144	40.2	2.82	5.69	8.51	78.8
	BF-Q-2	-4° C. filtrate	198	55.5	0.50	3.57	4.07	92.7
	BF-M	March, 1946	227	63.6	0.97	4.84	5.89	90.2

[*] Ether extractable material liberated by HCl treatment.

[†] 7-day pooled samples.

[‡] U. S. Army Quartermaster Corps.

fatty acids. The latter analysis was made as described previously. The results of these analyses are given in table 2. The values for the per cent absorption of the various fats are not corrected for "metabolic" fat excretion.

DISCUSSION

Separation of butterfat into two fractions by means of cold crystallization from an acetone solution yielded in Expt. 1 a liquid fraction which allowed a very rapid rate of growth of rats when incorporated in a sucrose diet. During a 6-week period these animals averaged 4.9 g. per day, a rate of growth above that usually obtained with Sprague-Dawley rats fed similar synthetic type diets (4). The solid fraction of the butterfat proved to be very low in nutritional value, for the rats gained only an average of 2 g. per day over the 6-week period. From the iodine number of 27 which this fraction possessed, it is evident that unsaturated acids were present; however, these acids were not identified. It seems doubtful that linoleic acid deficiency could have caused the slow rate of growth which occurred even during the first week of the experiment. The fecal analyses show that the rat was able to hydrolyze the fat, but that the fatty acids liberated were not well absorbed. The melting point of 42° C. was not too high, for fairly good absorption occurred in subsequent experiments in which analogous fractions were employed.

The wide differences in nutritive value between the two fractions prepared from September, 1945, butter were not evident in any of the subsequent fractionation trials in which the butterfat used was prepared from butter obtained in June and December of 1945, and February, June, July, and September of 1946. In most cases, however, the rats fed the butterfat fraction 1 ration grew at a rate slightly inferior to that of the control groups, and they excreted more total ether-extractable material in their feces. Growth of the rats fed the BF–2 rations was uniformly good in all experiments and the per cent absorption of this fat was always above 89 per cent. Henry *et al.* (7) concluded that there was no significant difference between the nutritive value of two fractions of butterfat which were obtained by a procedure similar to the one described in this paper. The iodine numbers of their fractions are in close agreement with those given in table 1. It should be noted that the growth of the animals used by the English workers was far below the usual growth of the animals used in the present study; also, a different ration was used.

The growth of the rats fed the two corn oil fractions was nearly identical to that of the corn oil control groups, and the gain in weight by the animals receiving either of the treated fats was equal to that of those fed the nontreated fats. This latter finding is in disagreement with the observation of Jack *et al.* (8), who showed that the treatment of butterfat with hexane decreased its nutritive value.

No reason is apparent for the fact that only in the first experiment were significant differences between the nutritive value of the two fractions obtained. Perhaps a more detailed separation of butterfat will be needed to secure consistent results as far as an extremely active fraction is concerned. In view of the finding of Jansen's group of workers (1, 9) that vaccenic acid (11, 12-eladic acid) has growth-promoting properties, it would be of interest to analyze the various fractions for this compound.

SUMMARY

Butterfat prepared from butter made in various months of the year has been fractionated into two fractions by cold crystallization from an acetone solution. A liquid fraction obtained from September, 1945, butter allowed rats to grow at a superior rate, while a solid fraction prepared from this butter caused a very slow rate of growth. This phenomenon was not repeated to the same degree in subsequent trials using other samples of butter.

Corn oil was separated into two fractions by a similar procedure but rats grew equally well when fed either of these fractions or corn oil itself.

The treatment of either fat by solvent had no deleterious effect on its nutritive value.

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STUDIES ON KETOSIS IN DAIRY CATTLE. IX. THERAPEUTIC EFFECT OF ADRENAL CORTICAL EXTRACTS

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There are some similarities between ketosis in cattle and Addison's disease in humans. Both subjects exhibit hypoglycemia and hypersensitiveness to insulin injections. The symptoms in both often are apparent at glucose levels which do not produce symptoms in normal subjects. Also in both, the blood glucose response to epinephrine injections is slight. There are a number of dissimilarities, but the positive relationships appeared to warrant some preliminary studies as to the possible rôle of the adrenal cortex in the development of ketosis in cattle.

EXPERIMENTAL

Four cases of uncomplicated ketosis were selected for study. Adrenal cortical extracts (Wilson) were injected subcutaneously at frequent intervals over short periods of time. Insofar as was possible, the cows were maintained on their customary feeding and management regime. Blood samples for glucose and acetone body determinations were drawn at frequent intervals before and during the course of treatment. Observations also were made of the behavior of the cows during the experimental periods. Blood glucose and acetone bodies were determined by methods previously cited (2).

Cow D. E. 259 was under observation for 3 days before treatment was initiated. The blood glucose, which was already at a low level on the first day of observation, decreased still more, and the blood acetone bodies increased from an already high level of 38.0 mg. per cent (fig. 1). The cow had refused all grain and ate but sparingly of hay during this period. Twenty milliliters of Wilson's extract were injected the evening of the third day. The following morning the cow appeared ravenous, quickly consuming 10 lbs. of hay and 5 lbs. of concentrate. A full bucket of grain containing approximately 10 lbs. of concentrate was fed and eaten rapidly. The blood glucose increased rapidly within the next 24 hours. Concurrently, the blood acetone bodies decreased.

The cortical extract was injected again on the fourth and fifth days, but the cow went off feed, apparently the result of over-feeding. On the seventh day, when the blood glucose and acetone bodies indicated a relapse and the appearance of the animal had worsened, injections again were initiated. Six injections, totaling 200 ml., were given within a 3-day period. The appetite of the cow again improved markedly; the blood picture likewise im-

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proved. Milk production tended to parallel the blood sugar curve rather closely, increasing during both periods of treatment (fig. 1). The cow recovered without further treatment.

Cow D. E. 262, from which the data for figure 2 were obtained, also had shown symptoms of ketosis several days before treatment was initiated. This animal had been off feed for several days, exhibited incoordination and general paresis, and was becoming emaciated. Milk production increased at first and then began to decrease rapidly, apparently with the onset of ketosis (fig. 2).

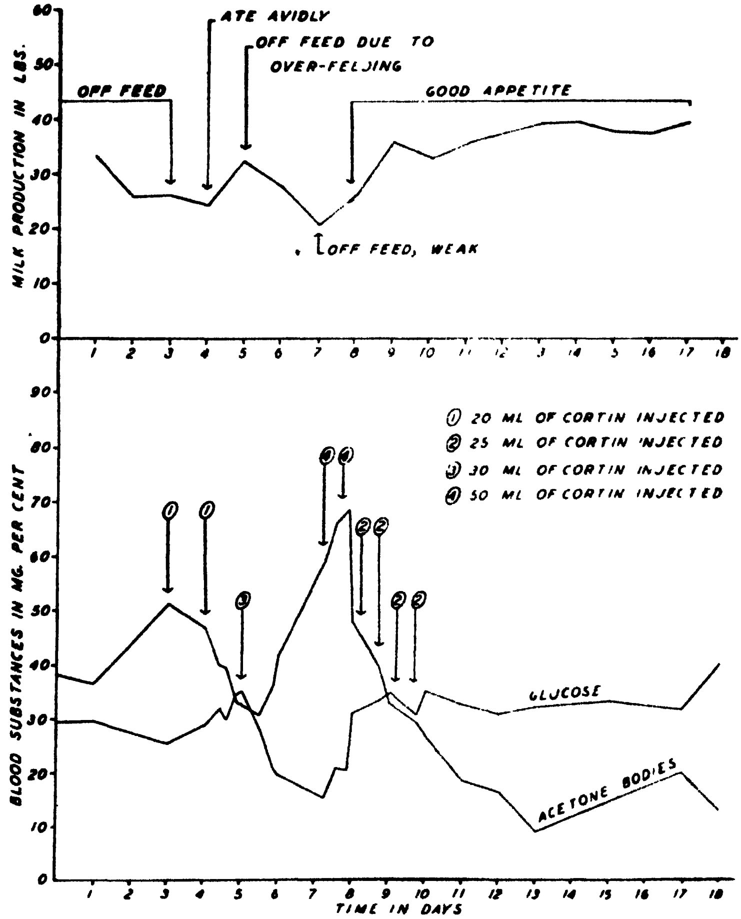

FIG. 1. Ketosis—cortin therapy (Cow D. E. 259).

During a 3-day period, 375 ml. of Wilson's adrenal cortical extract were injected subcutaneously. An improvement in appetite was observed within 24 hours. The blood picture also improved, the blood glucose and acetone bodies approaching normal levels within 5 days. Within 4 days of the last

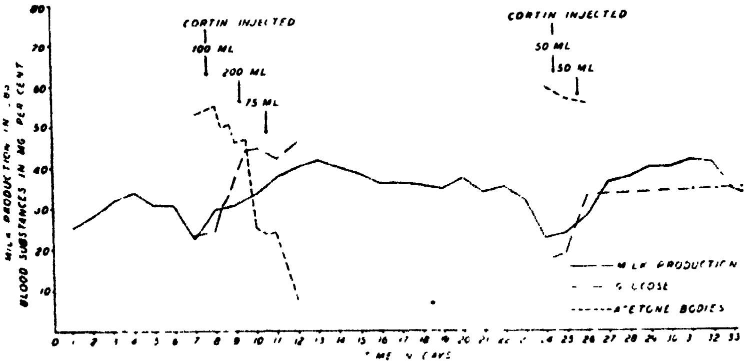

FIG. 2. Ketosis—cortin therapy (Cow D. E. 262).

injection, milk production, which had almost doubled following the initial injections, again began to decrease, indicating a relapse. Once more the animal refused all feed. An injection of 50 ml. of the adrenal cortical extract was made and repeated on the following day. Improvement in appetite, milk production, blood glucose, and blood acetone bodies was prompt. A week later, when the appearance of the animal denoted a possible relapse, glucose was injected intravenously.

In figures 3 and 4, data are presented graphically on the response to injections of adrenal cortical extracts of two cows exhibiting less advanced stages of ketosis. In both cases the ketonemia and hypoglycemia were marked, and both animals exhibited inappetence. The response to injections of the ad-

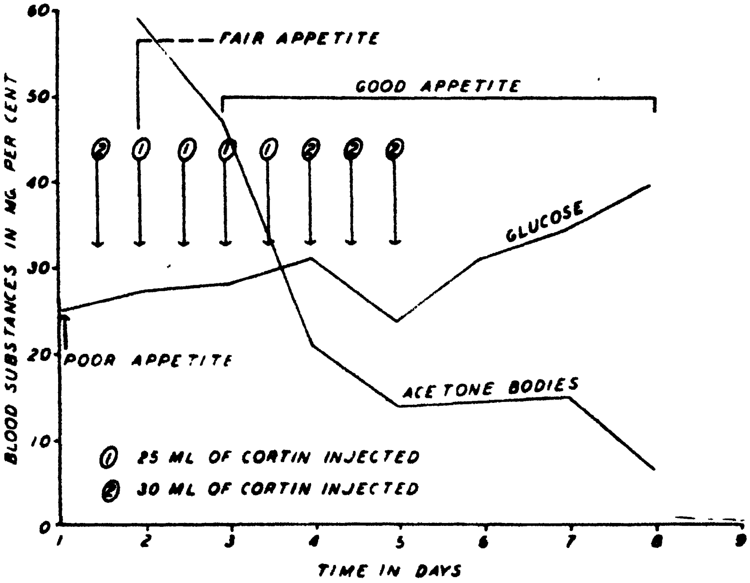

FIG. 3. Ketosis—cortin therapy (Cow D. E. 264).

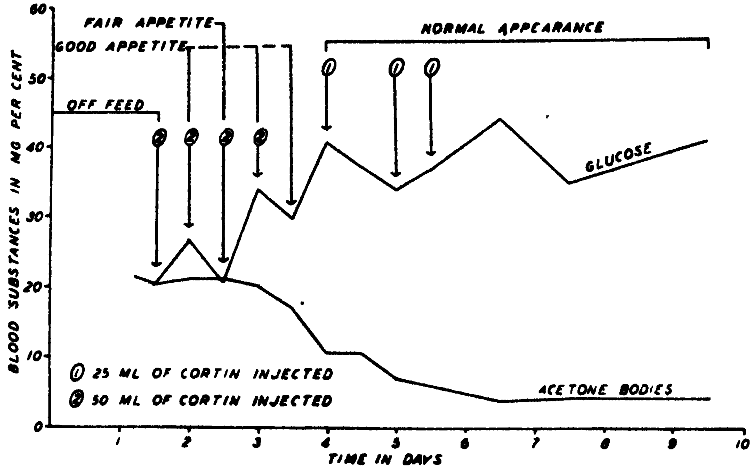

FIG. 4. Ketosis—cortin therapy (Cow D. E. 267).

renal cortical extract was prompt in the two animals. The appetite and general appearance of the two cows appeared normal within 3 to 4 days. No further treatment of any kind was required.

DISCUSSION

Caution must be exercised in drawing conclusions as to the effectiveness of any substance in promoting recovery of cows suffering from ketosis, since they recover from time to time without treatment and without any apparent change in management and feeding (2). The marked improvement following each injection period in each of the four cases studied appears to be significant however. It appears unlikely that four such cases selected at random all would improve so quickly purely by chance happening. Rather, it appears that the adrenal cortical extract did have a specific effect in promoting recovery.

This does not necessarily mean that ketosis in cattle is due to an adrenal insufficiency, since extracts of the adrenal cortex promote glyconeogenesis even in normal animals. The effect could have been strictly a therapeutic one in which improvement was due to an increased liver glycogen and/or blood glucose. There appeared to be little actual measurable increase in the blood sugar until after the appetite had improved, though it is possible that more carbohydrate was being made available to the body tissues.

The following observations on cows are similar to those observed in Addison's disease (3) and favor the view that an adrenal insufficiency may be involved: (a) Ketosis in dairy cows is a hypoglycemic condition. (b) There is a definite lowering of the glucose threshold at which symptoms become manifest (1). (c) Adrenal cortical extracts have elicited a favorable response in each of the four cases studied. (d) Cows with ketosis are more sensitive to insulin injections than normal cows (unpublished). (e) The blood glucose response to adrenalin injections is slight (1).

The following are not in accord with the view that an adrenal insufficiency is involved in the development of ketosis in dairy cows: (a) The plasma sodium and potassium appear to be normal (unpublished). (b) The intravenous glucose tolerance curve of cows with ketosis (1) is not typical of that of adrenal insufficiency in other species (3). (c) Cows with mild cases of ketosis and many with relatively severe cases frequently recover following a single intravenous injection of glucose.

Preliminary work indicates that both the plasma sodium and potassium are normal, although more data are needed to establish this with certainty. The secretion of the carbohydrate principle could still be affected abnormally, of course, without affecting the sodium and potassium relationship.

CONCLUSIONS

Four cows with ketosis, when treated with an extract of the adrenal cortex, responded immediately with an improvement in appetite and a return of the blood glucose and acetone bodies toward the normal values. However, an adrenal insufficiency is not necessarily believed to be indicated.

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THE INFLUENCE OF A SYNTHETIC THYROPROTEIN WHEN FED TO DAIRY COWS OVER AN EXTENDED PERIOD[1]

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INTRODUCTION

The development *in vitro* of a highly active thyroprotein by Reineke and Turner (2) has made possible the determination, by a number of investigators, of the influence of thyroprotein on milk secretion in dairy cattle. The results of these investigations rather consistently have shown an increase in milk and milk-fat production accompanied by an increase in heart rate and a decrease in body weight.

The feeding of a moderate daily dose of thyroprotein during a 3-week period was found to increase the fat content without greatly augmenting milk production (1). The present study was made to determine the influence of feeding thyroprotein over an extended period.

EXPERIMENTAL PROCEDURE

Nine dairy cows in various stages of declining lactation and gestation were fed daily either 10 or 15 g. of thyroprotein (Protamone[2]), which was incorporated in the grain ration. During the entire feeding period, daily milk weights (milking twice daily) were recorded, and milk samples were taken on two consecutive days each month. Individual milk samples were tested for their butterfat content by the Babcock method. Body weights and heart rates (measured with a stethoscope) were determined on two consecutive days each month. The part of the lactation during which thyroprotein was fed is compared with a similar segment of either a previous or a subsequent lactation. In giving information on reproductive performance, the last breeding date is listed as the date of conception. In tables 1 and 2 are included results from five cows used in the first experiment (1).

EXPERIMENTAL RESULTS

Immediate changes in milk production following the feeding of thyroprotein. Nine cows that received 10 g. of thyroprotein showed an average initial increase of 7.6 per cent, whereas five cows fed 15 g. of thyroprotein increased 19.7 per cent in milk production. These results are summarized in table 1.

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[2] The Protamone was generously supplied by the Cerophyl Laboratories, Kansas City, Missouri, through the courtesy of Dr. W. R. Graham, Jr.

TABLE 1

Immediate changes in milk production following the feeding of thyroprotein

Cow no.	Av. daily production (lbs.) for 7 days prior to feeding	Av. daily production (lbs.) for highest 7 days after feeding	% change in production
		10 g./day	
H37	29.3	34.0	+16.0
492	23.5	25.8	+ 9.8
383	22.3	25.6	+14.8
H41	15.2	15.7	+ 3.3
627	26.1	28.4	+ 8.8
621	64.3	66.8	+ 3.9
378	31.0	34.4	+11.0
485	23.0	24.2	+ 5.2
611	31.7	31.7	0.0
Av.	29.6	31.8	+ 7.6
		15 g./day	
628	15.8	21.5	+36.1
619	17.7	26.6	+50.3
H41	27.3	32.3	+18.3
492	29.1	32.5	+11.7
642	27.4	27.5	+ 0.4
Av.	23.5	28.1	+19.7

Milk production following the withdrawal of thyroprotein from the ration. Thyroprotein was withdrawn from the ration of five cows that had been receiving 10 g. per day. The average daily milk production for the last 7 days of feeding was 25.0 lbs. as compared with 20.1 lbs. for the second 7-day period after withdrawal, a decrease of 19.5 per cent. The daily milk production of three cows receiving 15 g. of thyroprotein decreased from 14.8 lbs. to 9.8 lbs., a decrease of 34.2 per cent following the withdrawal of thyroprotein from the ration (table 2).

TABLE 2

Milk production following the withdrawal of thyroprotein from the ration

Cow no.	Av. daily production (lbs.) for last 7 days of feeding	Av. daily production (lbs.) for second 7-day period after withdrawal	% change in milk production
	10 g./day		
H37	34.0	29.0	−14.7
492	24.9	21.1	−15.3
383	22.9	16.8	−26.6
H41	14.6	10.3	−29.5
627	28.4	23.3	−18.0
Av.	25.0	20.1	−19.5
	15 g./day		
619	10.9	4.6	−57.8
628	10.9	9.8	−10.1
621	22.7	14.9	−34.4
Av.	14.8	9.8	−34.2

Milk production following the withdrawal and the refeeding of thyroprotein. To determine whether thyroprotein was exerting a stimulating influence on milk secretion after a prolonged feeding period, it was withdrawn from the ration of four cows for 7 days and then included again. In each instance, a sharp decline in milk production followed the withdrawal of thyroprotein. When thyroprotein again was fed, three of four cows attained the pre-withdrawal level of milk production. The cow (378) that failed to return to the pre-withdrawal level of milk production had been receiving 40 g. of thyroprotein daily, and when refed was given only 15 g. daily. These results are summarized in table 3.

TABLE 3

Milk production following the withdrawal and the subsequent feeding of thyroprotein

Cow no.	Av. daily production by 7-day periods	Thyroprotein fed daily
	(lbs.)	*(g.)*
619	23.9	15
	19.5 (15.9*)	None
	17.9	15
	24.0	15
378	13.9	40
	13.1	40
	9.8 (5.5*)	None
	8.0	15
	7.4	15
611	13.1	15
	10.2 (7.1*)	None
	10.2	15
	13.2	15
642	22.1	15
	19.3 (16.6*	None
	18.7	15
	22.1	15

* Lowest daily production for the period.

Influence of feeding thyroprotein from 3 to 17 months. A Holstein-Friesian cow (H–41; born 10–27–39; calved 2–7–43; conceived 4–27–43; aborted 8-month fetus 1–3–44; cow infected with *Brucella abortus*) received 15 g. of thyroprotein daily for 2.5 months. Milk production increased from 27.3 lbs. to 32.3 lbs. (table 1) and the butterfat percentage increased from 3.11 to 4.32 in the first month of the feeding period. Body weight increased during thyroprotein feeding, and heart rate showed a maximum increase of 16 beats per minute. Increasing the dose of thyroprotein from 15 to 30 g. for 15 days did not prevent the decline in milk production which was occurring at that time. Body weight had increased by 106 lbs. and heart rate had decreased by 14 beats per minute 22 days after the removal of thyroprotein from the ration. These results are summarized in table 4. In 3 months of a control lactation cow H–41, calving at 4 years and 2 months,

TABLE 4

Influence of thyroprotein in the ration of cow H-41

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1943)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
Feb. (19 days)	56.7					
March	68.9	2.43	3.53			
April	66.1	1.98	3.00			
May	64.6	1.74	2.70			
June	45.5	1.25	2.75			
July	34.1	1.06	3.11	1271	67	
Aug.	29.3	1.27	4.32	1306	80	15
Sept.	22.8	0.95	4.20	1377	83	15
Oct.	12.7	0.58	4.59	1374	82	15*
Nov. (6 days)	6.9			1480†	68†	

* Thirty grams of thyroprotein daily from Oct. 15 to Nov. 1.

† Determinations made 22 days following the removal of thyroprotein from the ration.

produced 1,853 lbs. of milk with a 3.58 per cent test; in a similar segment of a lactation following calving at 3 years and 3 months, she produced 1,984 lbs. of milk with a 4.33 per cent test when thyroprotein was fed.

An Ayrshire cow (485; born 5-18-38; calved 5-6-42; conceived 6-26-42; calved normally 3-28-43) was fed 10 g. of thyroprotein daily for 4 months. Milk production increased only slightly (table 1); however, the fat content of the milk increased markedly. Body weight decreased and then increased, and by the end of the feeding period the cow had gained 63 lbs. in body weight. Although heart rate varied, in 3 of the 4 months it was considerably higher than in the month prior to thyroprotein feeding. Table 5 presents a summary of these results. This cow calved at 3 years and 1 month

TABLE 5

Influence of thyroprotein in the ration of cow 485

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
May (20 days)	28.5					
June	26.0					
July	22.9	0.93	4.04			
Aug. (15 days)	23.3	0.92	3.94	1027	64	
Aug. (16 days)	24.0	1.10	4.60			10
Sept.	21.3	0.98	4.58	980	78	10
Oct.	16.6	0.85	5.14	1013	65	10
Nov.	12.2	0.61	5.00	1033	92	10
Dec. (20 days)	7.3	0.39	5.33	1090	86	10

and in a 4-month segment of her lactation she produced 2,492 lbs. of milk with a 4.14 per cent test. In a similar segment of a subsequent lactation, calving at 3 years and 11 months, she produced 2,255 lbs. of milk with a 4.80 per cent test when fed thyroprotein.

A Brown Swiss, first-calf heifer (628; born 8–22–39; calved 9–28–42; conceived 12–7–42; calved normally 9–16–43) received 15 g. of thyroprotein daily for 4 months. This heifer had declined considerably in milk production after calving and it was evident that she was increasing in body weight at the expense of milk production. Milk production increased from 15.8 lbs. to 21.5 lbs. per day (table 1). and in the first 3 months of thyroprotein feeding this animal produced more milk than she had in the 3 months prior to thyroprotein feeding. Although milk production did not decline sharply after the withdrawal of thyroprotein (table 2), there were other indications that the heifer had received stimulation from the thyroprotein in the last month of the trial. The butterfat content of the milk increased following the inclusion of thyroprotein in the ration and decreased when thyroprotein was removed. From the first month to the third month of thyroprotein feeding, there was a decrease of 104 lbs. in body weight. When the feeding of thyroprotein was discontinued, body weight increased. However, 15 days after the withdrawal of thyroprotein from the ration, body weight was still 50 lbs. below the weight prior to feeding. Heart rate increased by 17 beats per minute during the first month of the feeding period and, following thyroprotein withdrawal, a marked decrease in heart rate occurred. A summary of these results is given in table 6. This animal received thyroprotein during 4 months of her first lactation, having calved at 3 years and 1 month. In these 4 months she produced 2,274 lbs. of milk with a 5.21 per cent test. In a similar segment of her following lactation, having calved at 4 years and 1 month, she produced 1,788 lbs. of milk with a 5.07 per cent test.

TABLE 6

Influence of thyroprotein in the ration of cow 628

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
Oct. (27 days)	24.7	1.19	4.80			
Nov.	21.4	0.83	3.90			
Dec.	17.9	0.83	4.65		....	......
(1943)						
Jan.	17.3	0.81	4.70			
Feb.	16.2	0.75	4.64		55	
March	19.5	0.97	4.98	1187	72	15
April	20.7	1.11	5.36	1126	66	15
May	20.5	1.02	4.98	1083	93	15
June	13.8	0.78	5.65			15
July	9.5	0.48	5.07	1137	78	

A Brown Swiss cow (619; born 4–19–36; calved 12–2–42; conceived 2–13–43; calved 11–30–43, the calf being dead when first observed) received 15 g. of thyroprotein daily for 4.5 months. Milk production increased from 17.7 lbs. to 26.6 lbs. per day (table 1). Thyroprotein was removed from the ration in the second month of the feeding period, and the average daily milk production decreased from 10.9 lbs. to 4.6 lbs. The fat content of the milk varied greatly but this was more apparent than real, since the cow was of a nervous nature and frequently failed to milk-out completely. A decrease in body weight was followed by an increase, the increase occurring when milk production was decreasing rapidly. The heart rate was increased by thyroprotein feeding and it remained above the pre-feeding level during the entire feeding period. These results are summarized in table 7. In 4 months of a control lactation period, having calved at 5 years and 7 months, no. 619 produced 3,161 lbs. of milk testing 4.05 per cent. In a similar segment of a lactation period when thyroprotein was fed, this animal, calving at 6 years and 7 months, produced 2,341 lbs. of milk with a 4.00 per cent test.

TABLE 7

Influence of thyroprotein in the ration of cow 619

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	*(lbs.)*	*(lbs.)*	(%)	*(lbs.)*	*(per min.)*	*(g.)*
Dec. (27 days)	36.3	1.46	4.01			
(1943)						
Jan.	36.7	1.14	3.10			
Feb.	28.1	0.56	2.00			
March (13 days)	19.4	0.90	4.63	1222	59	
March (18 days)	21.0					15
April	25.2	0.99	3.92	1216	70	15
May	21.4	0.89	4.17	1141	71	15*
June	18.8	0.66	3.51			15
July	11.5	0.52	4.56	1178	75	15
Aug. (18 days)	6.1					

* Thyroprotein withdrawn from ration for 7 days.

An Ayrshire cow (492; born 1–11–39; calved 4–16–43; conceived 8–10–43; calved normally 5–19–44) received 15 g. of thyroprotein daily for 5.5 months. Milk production increased from 29.1 lbs. to 32.5 lbs. per day (table 1). The fat test increased from 3.28 per cent prior to thyroprotein feeding to 4.07 per cent in the third month of thyroprotein feeding. In the following 2 months the fat content of the milk decreased to the pre-feeding level, even though milk production was falling rapidly. The fat content of the milk showed an increase only when the stimulus to secrete milk was low. The decrease in the fat content of the milk was associated with an increase in body weight. There was an initial loss in body weight; however, in the fifth month of thyroprotein feeding, no. 492 was 100 lbs. above her pre-feeding weight. Heart rate increased and then decreased. Table 8 presents a summary of these results. Similar segments of 5 months each of two lactations were available for comparison. Calving at 4 years and 3 months, no. 492 produced 3,274 lbs. of milk with a 3.82 per cent fat test when thyroprotein was fed; calving at 5 years and 4 months, she produced 2,912 lbs. of milk with a 3.30 per cent fat test when thyroprotein was not fed.

A Jersey cow (378; born 1–5–39; calved 9–1–42; conceived 2–25–43; aborted 7-month fetus 9–23–43; cow infected with *Brucella abortus*) was fed 10 g. of thyroprotein daily for 4 months, after which increasing amounts were fed until a dose of 40 g. was attained in the sixth month of thyroprotein feeding. Milk production increased from 31.0 lbs. to 34.4 lbs. (table 1) soon after the inclusion of thyroprotein in the ration and then declined rather rapidly. However, this lack of persistency also was observed in a previous lactation. An attempt to prevent further decrease in milk production by increasing the thyroprotein dose did not meet with success. Butterfat percentage increased, but this increase probably was a result of the advance in the stage of lactation, since the fat content of the milk secreted during thyroprotein feeding was about the same as that secreted in a similar segment of a previous lactation. Body weight increased gradually until the last month of the feeding period. At that time body weight decreased slightly, and this decrease in body weight was accompanied by an increase in heart rate by 11 beats per minute. These results are summarized in table 9. Calving at 2 years and 8 months, no. 378 produced 2,757 lbs. of milk with a 3.97 per cent test in 6 months. In a similar segment of a subsequent lactation, calving at 3 years and 8 months, this animal produced 3,978 lbs. of milk with a 4.05 per cent test when thyroprotein was fed.

TABLE 8

Influence of thyroprotein in the ration of cow 492

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1943)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
April (11 days)	33.5					
May	39.6	1.36	3.45			
June	35.7	1.12	3.15			
July	30.9	1.01	3.28	1010	75	....
Aug.	31.0	1.18	3.80	989	82	15
Sept.	26.0	1.02	3.93	1006	76	15
Oct.	21.1	0.86	4.07	977	69	15
Nov.	19.6	0.74	3.75	1007	70	15
Dec.	9.4	0.30	3.22	1110	69	15
(1944)						
Jan. (13 days)	3.2	0.12	3.83			15

TABLE 9

Influence of thyroprotein in the ration of cow 378

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
Sept. (20 days)	33.6	1.21	3.60		.	.
Oct.	31.9	1.12	3.52	830	82	. .
Nov.	33.1	1.23	3.72	848	79	10
Dec.	26.8	1.10	4.12	859	82	10
(1943)						
Jan.	23.6	0.93	3.93	859	74	10
Feb.	18.8	0.82	4.38	878	74	10
March	15.5	0.66	4.26	889	76	*
April	13.6	0.57	4.22	872	87	*

* Thyroprotein gradually increased from 10 g. per day to 40 g. per day.

A Brown Swiss, first-calf heifer (642; born 11–8–40; calved 5–2–43; conceived 7–2–43; calved normally 5–11–44) was fed 15 g. of thyroprotein daily for 6 months. Milk production was not augumented (table 1); however, by the third month of thyroprotein feeding the fat test had increased from 3.51 to 4.12 per cent. In the second month of the trial, thyroprotein was withdrawn from the ration for 7 days. Milk production decreased from 22.1 lbs. to 18.7 lbs. (lowest daily production was 16.6 lbs.). The addition of thyroprotein to the ration resulted in the return of milk production to the prewithdrawal level (table 3). Increasing the grain allowance by 2 lbs. per day, in the fourth month of the feeding period, resulted in a further increase in the fat content of the milk. This increase in the fat content of the milk was accompanied by an increase in body weight. These results are presented in table 10. Similar segments of 6 months each, of two lactations, are avail-

TABLE 10

Influence of thyroprotein in the ration of cow 642

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1943)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
May (25 days)	33.1	1.16	3.50			
June	31.9	1.02	3.20	.		
July	28.4	1.00	3.51	927	77	. .
Aug.	25.6	0.98	3.84	910	63	15
Sept.	20.8	0.83	4.01	906	64	15*
Oct.	21.5	0.89	4.12	905	58	15
Nov.	21.7	0.99	4.58	972	59	15
Dec.	19.5	0.90	4.59	1027	61	15
(1944)						
Jan.	18.5	0.79	4.27	1088	62	15
Feb. (9 days)	15.6	. . .		...	..	.

* Thyroprotein withdrawn from ration for 7 days.

able for comparison. Calving at 2 years and 6 months, no. 642 produced 3,909 lbs. of milk with a 4.22 per cent fat test when thyroprotein was fed; calving at 3 years and 6 months, she produced 6,039 lbs. of milk with a 4.03 per cent fat test when thyroprotein was not fed.

A Brown Swiss cow (621; born 12–10–36; calved 8–6–42; conceived 11–25–42; calved normally 8–31–43) received 10 g. of thyroprotein daily for 4 months and then 15 g. daily for 4 months. There was an initial increase in milk production from 64.3 lbs. per day to 66.8 lbs. (table 1). Fat production in the fifth month of thyroprotein feeding (eighth month of lactation) was similar to that in the month of peak fat production, which was just prior to the addition of thyroprotein to the ration. The fat content of the milk did not increase until the second month of thyroprotein feeding. Body weight decreased slightly and then gradually increased. By the seventh month of the feeding period, this animal had not attained a body weight equal to that recorded in the month prior to thyroprotein feeding; at that time she was in the sixth month of pregnancy. There was no increase in heart rate. It is believed, however, that heart rate was higher than it would have been had thyroprotein not been fed, since heart rate decreased following the removal of thyroprotein from the ration. These results are summarized in table 11. No. 621, calving at 5 years and 8 months, produced 12,671 lbs. of milk (4.00 per cent test) in 8 months when thyroprotein was fed. In a similar segment of a subsequent lactation, calving at 6 years and 8 months, she produced 9,398 lbs. of milk (4.00 per cent test).

TABLE 11

Influence of thyroprotein in the ration of cow 621

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	*(lbs.)*	*(lbs.)*	(%)	*(lbs.)*	*(per min.)*	*(g.)*
Aug. (23 days)	56.9	2.03	3.56			
Sept.	61.3	2.15	3.51			
Oct.	66.4	2.27	3.42	1358	80	
Nov.	64.7	2.25	3.48	1342	78	10
Dec.	61.5	2.48	4.04	1302	75	10
(1943)						
Jan.	59.3	2.40	4.04	1297	76	10
Feb.	55.9	2.09	3.73	1364	75	10
March	53.9	2.20	4.09	1324	76	15
April	50.6	1.95	3.86	1339	77	15
May	43.2	1.94	4.49	1339	75	15
June	29.7	1.41	4.74			15
July (14 days)	16.7	0.82	4.88	1397	68	

A Brown Swiss cow (611; born 8–24–34; calved 5–14–42; not pregnant) received 10 g. of thyroprotein daily for 6.5 months and 15 g. daily for an additional 10.5 months. There was no increase in milk production, but there was a substantial increase in the fat content of the milk. Average daily fat production remained fairly constant during the first 9.5 months of the feeding period, after which it gradually decreased. Increasing the dose of thyroprotein from 10 to 15 g. per day did not increase either milk or milk-fat production. The withdrawal of thyroprotein from the ration for 7 days in the fourteenth month of the feeding period resulted in a sharp decline in milk production (table 3), indicating that thyroprotein had been stimulating milk production. The feeding of thyroprotein during the summer months did not prevent a decrease in fat test, although the fat tests probably were higher than they would have been had thyroprotein not been fed. Body weight and heart rate fluctuated considerably, the lowest body weight being recorded when the heart rate was highest. Body weight remained below the pre-feeding weight until the sixteenth month of the feeding period. At the end of the feeding period, at which time milk production was declining rapidly, the cow weighed 154 lbs. more than she weighed prior to the feeding of thyroprotein. A summary of these results is presented in table 12. It was possible to compare 305 days of the experimental lactation with a similar seg-

TABLE 12

Influence of thyroprotein in the ration of cow 611

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
May (9 days)	29.7					
June	28.1					
July	30.3	1.13	3.72			
Aug. (15 days)	32.5	1.25	3.85	1233	60	
Aug. (16 days)	31.2	1.22	3.92			10
Sept.	30.3	1.26	4.16	1171	63	10
Oct.	28.4	1.29	4.54	1169	59	10
Nov.	28.8	1.28	4.45	1180	72	10
Dec.	26.9	1.27	4.72	1192	60	10
(1943)						
Jan.	24.7	1.12	4.55	1185	58	10
Feb.	23.3	1.12	4.81	1208	53	10
March	23.5	1.15	4.90	1204	58	15
April	23.7	1.16	4.91	1195	66	15
May	23.9	1.14	4.78	1116	88	15
June	19.5	0.93	4.75			15
July	16.9	0.78	4.61	1159	87	15
Aug.	15.5	0.68	4.41	1182	59	15
Sept.	11.8	0.54	4.61	1207	59	15*
Oct.	12.6	0.60	4.77	1202	54	15
Nov.	12.1	0.59	4.89	1259	58	15
Dec.	8.2	0.39	4.80	1303	54	15
(1944)						
Jan. (11 days)	3.6			1387	64	15

* Thyroprotein withdrawn from ration for 7 days.

ment of a previous lactation. Calving at 6 years and 6 months, no. 611 produced 6,704 lbs. of milk with a 4.39 per cent test in 305 days of a normal lactation; calving at 7 years and 8 months, she produced 8,161 lbs. of milk with a 4.55 per cent test in 305 days when thyroprotein was fed.

DISCUSSION

The feeding of 10 g. of thyroprotein daily to dairy cows resulted in an initial increase in milk production of 7.6 per cent. Perhaps the most striking observation is the variation in response (from no response to + 14.8 per cent). One would anticipate, however, some variation in response, since the cows differed in age, stage of lactation, level of milk production, and breed. When 15 g. of thyroprotein were fed daily, the initial increase was more than twice as great as that obtained with 10 g. In addition, the variation in response also was greater, from + 0.4 to + 50.3 per cent.

The per cent decrease in milk production on the withdrawal of thyroprotein from the ration was greater than the per cent increase following the feeding of thyroprotein. The number of cows from which the averages were obtained, however, is not the same. Five cows that received 10 g. of thyroprotein daily and from which the thyroprotein eventually was removed showed an average increase of 11 per cent in milk production following feeding and an average decrease of 19.5 per cent following removal. In certain cases, after the withdrawal of thyroprotein from the ration, the level of milk production in the second week was lower than in the third week. In one instance (no. 383), the average daily milk production for the second 7-day period after withdrawal was 16.8 lbs. but in the third week after removal it was 18.4 lbs. The magnitude of the decrease in milk production following thyroprotein withdrawal appears to be associated with the stage of lactation and the degree of stimulation received during the feeding period. These observations suggest that, from the standpoint of milk and milk-fat production, after thyroprotein is included in the ration it should not be withdrawn until one desires to terminate lactation. Lactation will cease rapidly following thyroprotein withdrawal.

Of the nine cows receiving thyroprotein from 3 to 17 months, six weighed more and three weighed less at the end of the feeding period than at the beginning. In three instances in which observations were made, body weight increased rapidly following thyroprotein removal. Two of three cows that decreased in body weight during thyroprotein feeding failed to secrete milk with a higher fat content than that observed in a similar segment of a control lactation. The question may be raised as to whether it is undesirable to have cows continue to lose in body weight after the peak of lactation is passed. If this continued loss is undesirable, the additional feed intake required to prevent it must be determined. Since body weight increases rapidly following thyroprotein withdrawal, it should not be too difficult to attain the desired body weight before the next calving. Another point to consider would be whether the latter part of the lactation plus the dry period or the dry period alone is the most desirable time to replenish the body reserves of a cow.

Body weight losses may not be severe following thyroprotein feeding, but the fat content of the milk may decrease despite an initial increase (no. 492). Increasing the feed intake may result in a further increase in the fat content of the milk (no. 642). This suggests the desirability of increasing the feed intake of an animal when thyroprotein feeding is started if a maximum response is desired.

SUMMARY

The daily feeding of 10 g. of thyroprotein to nine cows resulted in an initial increase of 7.6 per cent in milk production; five cows receiving 15 g. of thyroprotein daily increased 19.7 per cent in milk production.

The average daily milk production in the second week after the withdrawal of thyroprotein was 19.5 per cent below that of the last week of thyroprotein feeding in five cows receiving 10 g. of thyroprotein; in three cows receiving 15 g. of thyroprotein the decrease was 34.2 per cent.

In three of four cows receiving thyroprotein, the withdrawal and refeeding of thyroprotein resulted in a marked decrease in milk production and then a return to the pre-withdrawal level. The cow not returning to the pre-withdrawal level was not fed thyroprotein at the previous level.

Of nine dairy cows that received either 10 or 15 g. of thyroprotein daily for periods varying from 3 to 17 months, six secreted milk with a higher fat content and six produced more milk than that secreted in a similar segment of either a previous or a subsequent lactation. Of the three cows not showing an increase in milk production during the entire thyroprotein feeding period, only one failed to show an initial response in milk production.

The author is indebted to Dr. H. J. Metzger of the New Jersey Agricultural Experiment Station for the determination of *Brucella abortus* in two of the animals.

REFERENCES

(1) **Reece, R. P.** The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows over a Three-week Period. Jour. Dairy Sci., 27(7): 545–550. 1944.

(2) **Reineke, E. P., and Turner, C. W.** Formation in Vitro of Highly Active Thyroproteins. Their Biologic Assay, and Practical Use. Mo. Agr. Expt. Sta. Res. Bul. 355. 1942.

ASSOCIATION ANNOUNCEMENTS

PROGRAM

FORTY-SECOND ANNUAL MEETING
OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

ONTARIO AGRICULTURAL COLLEGE
GUELPH, ONTARIO, CANADA
JUNE 24-26, 1947

PROGRAM COMMITTEE

GENERAL:
- J. A. NELSON, *Chairman*
  Montana State College
- G. E. RAITHBY
  Ontario Agricultural College

MANUFACTURING:
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- C. L. HANKINSON
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- G. H. WISE
  Iowa State College
- G. W. SALISBURY
  Cornell University

REGISTRATION

ADMINISTRATION BUILDING
ONTARIO AGRICULTURAL COLLEGE

Meetings will be held in the buildings on the campus of Ontario Agricultural College. Headquarters will be in the Administration Building.

PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for projection of standard and 2″ x 2″ slides. Projectors for 16 mm. movies will be available by arrangement. Request for projection equipment should be made at the time abstracts of papers are submitted to the respective section Chairman. For the benefit of anyone bringing special electrical equipment, the available current is all 25 cycle.

COMMITTEE MEETINGS

Those wishing rooms for Extension and Production Section Committee meetings should write or contact G. E. Raithby and those in the Manufacturing Section wishing the use of rooms for Committee meetings should write or contact W. H. Sproule.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings before, during, or after the regular session will please contact G. E. Raithby. Provision also can be made for a limited number of breakfasts, luncheons, or dinners for special groups.

SCHEDULE OF PROGRAMS

Date and Time	General	Manufacturing	Production	Extension
Monday *June 23, 1947*	Registration			
Tuesday *June 24, 1947*				
8:00	Registration			
9:30–12:00	Opening Session			
1:00– 4:00		Papers	Papers	Papers
4:00– 5:00	Committees	Committees	Committees	Committees
8:00	Reception (Informal)			
Wednesday *June 25, 1947*				
9:00–12:00		Papers	Papers, A & B	Session
1:00– 1:30	Group Picture			
1:30– 5:30			Joint Meeting and Symposium	
1:30– 3:30		Papers		
3:30– 4:30		Business		
4:30– 5:30		Committees		
8:00	Mixer			
Thursday *June 26, 1947*				
9:00–11:00		Papers	Papers	Papers
11:00–12:00		Business	Business	Business
1:00– 3:00		Invitational Papers	Papers, A & B	
1:00– 2:00				Papers
2:00– 3:00				Business
3:00– 5:00	Business			
6:30	Annual Banquet			

GENERAL PROGRAM

Tuesday, June 24, 1947

Eastern Daylight Saving Time

9:30–12:00 Opening Sessions, *War Memorial Hall*
G. E. Raithby, *Department of Animal Husbandry,* presiding
Introduction of Officers and Guests
Address of Welcome
W. R. Reek, *Acting President of Ontario Agricultural College*
Presidential Address
Fordyce Ely, *President, American Dairy Science Association*
Guest Speaker
Hon. George A. Drew, *K. C., Premier of the Province of Ontario*
Announcements
1:00– 4:00 Sectional Meetings
Joint Meeting Production Sections A & B
Field Husbandry Building
Manufacturing Section
Dairy Building
Extension Section
Animal Husbandry Building
4:00– 5:00 Committee Meetings
8:00 Reception (Informal)

Wednesday, June 25, 1947

9:00–12:00 Sectional Meetings
Production Section A
Field Husbandry Building
Production Section B
Field Husbandry Building
Manufacturing Section
Dairy Building
Extension Section
War Memorial Hall
1:00– 1:30 Group Picture, *Administration Building*
1:30– 5:30 Sectional Meetings
Joint Meeting of Production Sections A & B and Extension Section
War Memorial Hall
Manufacturing Section
Dairy Building

3:30– 4:30 Business Meeting
Manufacturing Section
4:30– 5:30 Committee Meetings
Manufacturing Section
8:00 Mixer

Thursday, June 26, 1947

9:00–11:00 Sectional Meetings
Joint Session Production Sections A & B
War Memorial Hall
Manufacturing Section
Dairy Building
Extension Section
Animal Husbandry Building
11:00–12:00 Business Meeting of Sections
Production Section
War Memorial Hall
Manufacturing Section
Dairy Building
Extension Section
Animal Husbandry Building
1:00– 3:00 Sectional Meetings
Production Section A
Field Husbandry Building
Production Section B
Field Husbandry Building
Manufacturing Section
Dairy Building
Extension Section
Animal Husbandry Building
3:00– 5:00 General Business Session
War Memorial Hall
6:30 Annual Banquet
Creelman Hall
Installation of Officers and Presentation of American Dairy Science Association and Borden Awards

MANUFACTURING PROGRAM

Tuesday, June 24

Afternoon Session

Dairy Building

C. L. HANKINSON, *Chairman*

1:00– 4:00 **Bacteriology**

M1 Application of the Phosphatase Test to Various Dairy Products. GEORGE P. SANDERS AND OSCAR S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

M2 Time-Temperature Conditions Required to Inactivate Phosphatase in Different Dairy Products. GEORGE P. SANDERS AND OSCAR S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

M3 Determining the Germicidal Potency of Quaternary Ammonium Compounds and Their Use in Dairy Sanitation. W. S. MUELLER, D. B. SEELEY, AND E. P. LARKIN, *Massachusetts State College.*

M4 Effectiveness of Hypochlorite and Quaternary Ammonium Germicides in Destroying *Streptococcus agalactiae* in a Mastitis Sanitation Procedure. K. R. SPURGEON, P. R. ELLIKER, W. J. HARPER, AND J. R. FROEDGE, *Purdue University.*

M5 The Resazurin Reductase Test Using Prepared Sterile Dry Vials. N. S. GOLDING, *State College of Washington.*

M6 Some Observations on the Inversion of Sucrose by Invertase. L. E. MULL AND L. R. ARRINGTON, *University of Florida.*

M7 Observations on the Microscopic Analysis of Raw and Pasteurized Milk. G. H. WATROUS, JR., *Pennsylvania State College.*

M8 An Activity Test for Cheddar and Cottage Cheese Starters. B. E. HORRALL AND P. R. ELLIKER, *Purdue University.*

M9 Special Cultures for Manufacture of Blue Cheese from Pasteurized Milk. C. E. PARMELEE AND F. E. NELSON, *Iowa State College.*

M10 Lipase Production by *Mycotorula lipolytica.* I. I. PETERS AND F. E. NELSON, *Iowa State College.*

4:00– 5:00 **Committee Meetings**

Wednesday, June 25
Morning Session
Dairy Building
C. L. Hankinson, *Chairman*

9:00–12:00 **Dry Milk Powders and Cheese**

M11 The Vapor Pressure–Moisture Relationships in Dry Milk Products. R. W. Kunkel and S. T. Coulter, *University of Minnesota.*

M12 Some Factors Influencing the Design of Spray Driers. Arnold Kitzer and S. T. Coulter, *University of Minnesota.*

M13 Changes Produced in Milk on Heating. H. A. Harland, R. Jenness, and S. T. Coulter, *University of Minnesota.*

M14 Some Changes in Dry Whole Milk during Storage. R. Jenness, S. T. Coulter, H. A. Harland, and L. K. Crowe, *University of Minnesota.*

M15 The Relationship of Ascorbic Acid to the Keeping Quality of Dry Whole Milk. S. T. Coulter and R. Jenness, *University of Minnesota.*

M16 Factors Affecting the Ease of Reconstitution of Milk Powders. U. S. Ashworth and H. A. Bendixen, *State College of Washington.*

M17 The Use of Non-fat Dry Milk Solids for Mother and Batch Starters. B. E. Horrall and P. R. Elliker, *Purdue University.*

M18 Influence of Per Cent Oxygen in the Headspace Gas of Container on the Quality of Dry Whole Milk during Storage for One Year. G. H. Wilster, *Oregon State College.*

M19 A Comparison of the Yields of Cheddar Cheese Obtained from Raw, Holder Pasteurized and High Short-Time Pasteurized Milk. O. W. Irvine, L. R. Bryant, D. C. Hill, and W. H. Sproule, *Ontario Agricultural College.*

Wednesday, June 25
Afternoon Session
Dairy Building
B. E. Horrall, *Chairman*

1:30– 3:30 **Chemistry**

M20 A Study of the Volatile Acidity in Milk. P. G. Miller, P. L. Zimmerman, and E. B. Oberg, *Carnation Company, Milwaukee, Wisconsin.*

M21 Some Unique Properties of Lactose as a Dietary Carbohydrate. LLOYD K. RIGGS, *Kraft Foods Company, Chicago, Illinois.*

M22 The Relationship between the Oxidation of Ascorbic Acid and the Development of the Oxidized Flavor in Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*

M23 The Use of Carotene for Coloring Butter. G. A. RICHARDSON AND M. L. LONG, *University of California.*

M24 Graphic Procedure for Determining Quantitative Results of Volumetric Analysis. W. I. TRETSVEN, *Advisory Service, Chicago, Illinois.*

M25 Some Chemical Reactions Involved in the Production of the Sunlight Flavor in Milk. D. G. KEENEY AND D. V. JOSEPHSON, *Ohio State University.*

M26 A New Method for Determining Concentration of Quaternary Ammonium Germicide Solutions. W. J HARPER AND P. R. ELLIKER, *Purdue University,* AND W. K. MOSELEY, *Indianapolis, Indiana.*

M27 Ionic Exchangers in the Dairy Industry. O. F. GARRETT, *M and R Dietetic Laboratories, Columbus, Ohio.*

3:30- 4:30 **Business Session**

4:30- 5:30 **Committee Meetings**

Thursday, June 26

Morning Session

Dairy Building

C. L. HANKINSON, *Chairman*

9:00–11:00 **Ice Cream, Evaporated Milk, and Sanitation**

M28 A Microscopic Study of the Texture of Ice Cream, W. S. ARBUCKLE, *University of North Carolina.*

M29 The Place of the Private Quality Control Laboratory in the Dairy Industry. M. A. COLLINS, *Dryden, New York.*

M30 Newly Developed Cleaning Aids for the Dairy Industry. JOHN R. PERRY, *Sealtest, Inc., New York, N. Y.*

M31 Some Observations on the Role of Sulphydryls in Heated Milk. STUART PATTON AND D. V. JOSEPHSON, *Ohio State University.*

M32 The Utilization of the Mineral-Ion Exchange Principle in the Manufacture of Evaporated Milk. D. V. JOSEPHSON AND C. B. REEVES, *Ohio State University.*

M33 The Manufacture of High-Solids Evaporated Milk. MARK KEENEY AND D. V. JOSEPHSON, *Ohio State University.*

M34 Condensing Whole Milk for Ice Cream Mix with the Vacreator. G. H. WILSTER, *Oregon State College.*

M35 A Study of the Use of Nordihydroguairaitic Acid in the Storage of Frozen Sweet Cream. J. W. STOLL, E. O. HERREID, AND P. H. TRACY, *University of Illinois.*

11:00–12:00 **Business Session**

Thursday, June 26

Afternoon Session

Dairy Building

C. L. HANKINSON, *Chairman*

1:00– 3:00 **Special Invitational Papers**

Utilization of Whey. B. H. WEBB, *Bureau of Dairy Industry, U.S.D.A.*

Milk Lipase. I. A. GOULD, *Dairy Department, University of Maryland.*

Continuous Methods for the Manufacture of Butter. A. W. FARRALL, *Agricultural Engineering, Michigan State College.*

Physical Chemistry as Applied to Ice Cream Manufacture. H. H. SOMMER, *University of Wisconsin.*

3:00– 5:00 **General Business Session**
War Memorial Hall

6:30 **Annual Banquet**
Creelman Hall

PRODUCTION PROGRAM

Tuesday, June 24

Afternoon Session

Field Husbandry Building

DWIGHT M. SEATH, *Chairman*

1:00– 4:00 SECTIONS A & B, **Artificial Breeding, Heredity Studies, Cross Breeding**

P1 The Influence of Streptomycin upon the Livability and Bacterial Content of Bull Semen. J. O. ALMQUIST, W. T. S. THORP, AND P. J. GLANTZ, *Pennsylvania State College.*

P2 Some Effects of Adding Thyroxine to Bull Semen. A. B. Schultze and H. P. Davis, *University of Nebraska.*

P3 Total Digestible Nutrients and Protein Levels for Dairy Bulls Used in Artificial Insemination. Cecil Branton, R. W. Bratton, and G. W. Salisbury, *Cornell University.*

P4 A Study of Factors Affecting the Length of Gestation in Dairy Cattle. H. A. Herman and R. W. Spalding, *University of Missouri.*

P5 The Relationships of Frequency of Ejaculation, Age and the Ratio of Plasma Calcium and Phosphorus Levels to the Plasma Phosphatases of Dairy Bulls. J. T. Reid, G. M. Ward, R. L. Salsbury, and C. E. Shuart, *New Jersey Agricultural Experiment Station.*

P6 Light Variation Associated with Conception Rate in Artificial Breeding. Ernest Mercier and G. W. Salisbury, *Animal Industry Service, Department of Agriculture of Quebec, Canada, and Cornell University.*

P7 Studies of Oxidative Mechanisms in Bull Semen. J. T. Reid, R. L. Salsbury, and G. M. Ward, *New Jersey Agricultural Experiment Station.*

P8 Collecting Genetic Data through Cooperating Dairymen. N. P. Ralston, S. W. Mead, and W. M. Regan, *University of California.*

P9 Variation in the Type Ratings of Individual Ayrshire Cows. George Hyatt, Jr., and W. J. Tyler, *West Virginia University.*

P10 Heritability of Type Ratings, and the Correlation between Type and Butterfat Production of Ayrshire Cows. W. J. Tyler and George Hyatt, Jr., *West Virginia University.*

P11 Analysis of the Production Records of Cross-Bred Dairy Cattle. R. A. Hilder and M. H. Fohrman, *Bureau of Dairy Industry, U.S.D.A.*

P12 Progress Report on Cross-Breeding of Dairy Cattle at Beltsville. M. H. Fohrman, *Bureau of Dairy Industry, U.S.D.A.*

P13 A Case of Intersex in Dairy Cattle. W. W. Yapp, *University of Illinois.*

4:00– 5:00 Committee Meetings

Wednesday, June 25

Morning Session

Field Husbandry Building

DWIGHT M. SEATH, *Chairman*

9:00–12:00 SECTION A, **Feeding and Management**

P14 Dairy Cattle Improvement Work of the Imperial Agricultural Research Institute—India. J. D. S. KUMARAN, *University of Missouri.*

P15 The Use of the Pen Barn as a Means of Mastitis Control. P. L. KELLY, D. F. BREAZEALE, G. S. HARSHFIELD, AND A. B. HOERLEIN, *South Dakota State College.*

P16 Report on Attempts to Prevent Mid-Summer Slump in Milk Production by Hay Feeding. D. M. SEATH AND G. D. MILLER, *Louisiana State University.*

P17 The Influence of Cracked Soybeans and Soybean Hay on the Flavor and Quality of Milk. ERLE E. BARTLEY AND C. Y. CANNON, *Iowa State College.*

P18 The Value of Adding Ground Alfalfa Hay to the Concentrate Mixture Fed with Prairie Hay in Rations for Dairy Cows. A. H. KUHLMAN AND H. W. CAVE, *Oklahoma A. & M. College.*

P19 The Effects of Calcium and Other Mineral Elements on the Lipid Partition in the Feces of Milking Cows. G. M. WARD AND J. T. REID, *New Jersey Agricultural Experiment Station.*

P20 Cobalt Tolerance in Young Dairy Cattle. H. A. KEENER, G. P. PERCIVAL, AND K. S. MORROW, *University of New Hampshire,* AND G. H. ELLIS, *U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y.*

P21 Phosphorus Metabolism Studies. I. Secretion of Phosphorus in Milk as Determined with the Radioactive Isotope. C. L. COMAR, W. A. KRIENKE, P. T. DIX ARNOLD, R. B. BECKER, AND GEORGE K. DAVIS, *University of Florida.*

P22 Feeding Value and Digestibility of Dehydrated Sweet Potatoes. L. L. RUSOFF, D. M. SEATH, AND G. D. MILLER, *Louisiana State University.*

George Wise, *Chairman*

9:00–12:00 Section B, **Colostrum, Mastitis, Milk Secretion**

P23 Studies on the Globulins of Bovine Colostrum. I. Isolation and Properties of a Water Soluble Globulin. R. G. Hansen and P. H. Phillips, *University of Wisconsin.*

P24 Studies on the Globulins of Bovine Colostrum. II. The Absorption of Globulins by the Young Calf. R. G. Hansen and P. H. Phillips, *University of Wisconsin.*

P25 Tocopherol Levels in the Colostrum and in the Early Milk of the Dairy Cow. D. B. Parrish, G. H. Wise, and J. S. Hughes, *Kansas State College.*

P26 Action of Bacterial Filtrates Infused in the Mammary Gland. Max L. Dawdy and W. E. Petersen, *University of Minnesota.*

P27 The Effect of Intramammary Treatment for Mastitis upon Milk Production. Eric W. Swanson and H. A. Herman, *University of Missouri.*

P28 The Use of Penicillin vs. Natural Recovery as a Means of Treatment of Mastitis. D. F. Breazeale, P. L. Kelly, G. S. Harshfield, and A. B. Hoerlein, *South Dakota State College.*

P29 The Use of Non-Chlorine Disinfectants for the Washing of Cows' Udders. Robert H. Keith and Paul M. Reaves, *Virginia Polytechnic Institute.*

P30 The Effect of Reduced Feed Intake on Mammary Growth and Lactation. J. F. Sykes, T. R. Wrenn, and S. R. Hall, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P31 Further Studies on the Effect of Vitamin D on Some of the Blood Changes in Normal and Milk Fever Cows at Parturition. J. W. Hibbs, W. D. Pounden, and W. E. Krauss, *Ohio State University.*

P32 The Effect of the Quality and Quantity of Feed Prepartum and Post-partum upon Blood Glucose and Blood Acetone Bodies. J. C. Shaw and G. M. Cairns, *University of Maryland.*

P33 Vitamin A Deficiency in Dairy Cattle on Rations Containing Ground Raw Soybeans. J. C. Shaw, *University of Maryland,* and L. A. Moore and J. F. Sykes, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

Wednesday, June 25
Afternoon Session
War Memorial Hall

W. T. Crandall and D. M. Seath, *Co-chairmen*

1:30– 5:30 **Joint Meeting with Extension Section**

Report of Dairy Cattle Health Committee, C. G. Bradt, *Chairman.*

Symposium—Brucellosis in Cattle.

3:00– 3:30 **Recess**

Report of Dairy Cattle Breeding Committee. E. J. Perry, *Chairman.*

Report of Breeds Relations Committee. H. A. Herman, *Chairman.*

Symposium with Purebred Dairy Cattle Association participating, Getting the Most Out of Our Nation-Wide Testing Program.

Thursday, June 26
Morning Session
War Memorial Hall

Dwight M. Seath, *Chairman*

9:00–11:00 Sections A & B, **Roughage and Pasture**

P34 Values of Regular Corn Silage, Grainless Corn Silage and Ear Corn Silage in the Dairy Ration. Kenneth M. Dunn, Ray E. Ely, and Carl F. Huffman, *Michigan State College.*

P35 Carotene Losses from Artificially Dehydrated Alfalfa and from Artificially Dehydrated Alfalfa Silage. R. G. Washburn, W. E. Krauss, and C. F. Monroe, *Ohio State University.*

P36 Silage Density: Effect of Pressure, Time and Crop Condition. A. E. Perkins and R. G. Washburn, *Ohio State University.*

P37 The Comparative Feeding Value of Corn Silage and Corn-Treated Meadow Crop Silage, with and without the Addition of Dilute Acetic Acid. C. F. Monroe, A. E. Perkins, C. E. Knoop, and R. C. Thomas, *Ohio State University.*

P38 The Digestibility of Coarsely Ground and Finely Ground Alfalfa for Dairy Heifers. Eric W. Swanson and A. C. Ragsdale, *University of Missouri.*

P39 Drying Requirements, Losses Due to Drying and Quality of Forage Obtained in Mow Hay Drying Experiments at Beltsville. J. B. SHEPHERD, L. G. SCHOENLEBER, H. G. WISEMAN, AND C. G. MELIN, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P40 Grazing Management Studies of Orchard Grass-Ladino Clover. P. S. WILLIAMS, V. G. SPRAGUE, C. B. KNODT, AND K. M. AUTREY, *Pennsylvania State College, and U. S. Regional Pasture Laboratory, State College, Pa.*

P41 The Evaluation of Several Grass-Legume Mixtures for Grass Silage and Aftermath Grazing. C. B. KNODT, V. G. SPRAGUE, P. S. WILLIAMS, AND K. M. AUTREY, *Pennsylvania State College, and the U. S. Regional Pasture Laboratory, State College, Pa.*

11:00–12:00 **Business Meeting**

Thursday, June 26

Afternoon Session

Field Husbandry Building

DWIGHT M. SEATH, *Chairman*

1:00– 3:00 SECTION A, **Milk Secretion**

P42 The Influence of Thyroprotein in the Ration of Dairy Cattle. RALPH P. REECE, *New Jersey Agricultural Experiment Station.*

P43 Some Effects of Feeding Thyroprotein to Dairy Cows. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P44 The Effect of Thyroprotein and Feed Intake on the Heart Rate of Dairy Steers. J. F. SYKES, T. R. WRENN, L. A. MOORE, AND J. W. THOMAS, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P45 Further Observations on the Effects of Feeding Thyroprotein to Dairy Cows. A. H. VAN LANDINGHAM, GEORGE HYATT, CHARLES E. WEAKLEY, JR., AND H. O. HENDERSON, *West Virginia University.*

P46 Absorption and Elimination of Thiouracil in Ruminants. RAY E. ELY, KENNETH J. OLSON, AND E. P. REINEKE, *Michigan State College.*

P47 The Influence of Thiouracil on Mammary Lobule-Alveolar Growth in Mice. JOHN P. MIXNER, *New Jersey Agricultural Experiment Station.*

P48 Factors Influencing the Male Hormone Content of Cow Manure. C. W. TURNER, *University of Missouri.*

P49 Progress Report in Study of Certain Goitrogens. G. W. PIPES AND C. W. TURNER, *University of Missouri.*

P50 The Effect of the Interval between Washing of the Udder and Attachment of Milking Machines upon the Bacterial Flora and Milk Production of Dairy Cows. C. B. KNODT, J. J. REID, P. S. WILLIAMS, AND E. M. KESLER, *Pennsylvania State College.*

GEORGE WISE, *Chairman*

1:00– 3:00 SECTION B, **Calf Feeding**

P51 The Riboflavin Content of Cow's Colostrum. T. S. SUTTON AND HAROLD E. KAESER, *Ohio State University.*

P52 Some Possible Relationships between Management, Fore-Stomach Contents, and Diarrhea in the Young Dairy Calf. W. D. POUNDEN AND J. W. HIBBS, *Ohio State University.*

P53 The Effect of Feeding Various Levels of Vitamin A on the Depletion Time of Dairy Calves. W. C. JACOBSON, H. T. CONVERSE, AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P54 The Antirachitic Properties for Calves of Hay Harvested by Field Curing, Barn Drying and Making Wilted Silage. L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P55 Changes in the Cell Volume and in the Concentration of Several Inorganic Constituents in the Blood of the Dairy Calf during Its Early Post-Natal Development. G. H. WISE, M. J. CALDWELL, D. B. PARRISH, AND J. S. HUGHES, *Kansas State College.*

P56 Distillers' Dried Solubles and Grains with Solubles as a Supplement in Dairy Calf Rations. J. R. SCHABINGER AND C. B. KNODT, *Pennsylvania State College.*

P57 The Utilization of β-Carotene, Vitamin A Alcohol, and the Natural Ester of Vitamin A by Holstein Heifers. R. H. ROSS, C. B. KNODT, AND N. B. GUERRANT, *Pennsylvania State College.*

P58 Soybean Oil Filled Milks for Feeding Young Dairy Calves. NORMAN JACOBSON AND C. Y. CANNON, Iowa *State College.*

P59 The Placental Transfer and Colostral Storage of Vitamin D in the Bovine. H. D. EATON, A. A. SPIELMAN, AND J. K. LOOSLI, *Cornell University.*

P60 The Effect of Protein Level on the Nitrogen Metabolism and Gains in Weight of Growing Holstein Calves. G. P. LOFGREEN AND J. K. LOOSLI, *Cornell University.*

3:00– 5:00 **General Business Session**
War Memorial Hall

6:30 **Annual Banquet**
Creelman Hall

EXTENSION PROGRAM

Tuesday, June 24

Afternoon Session

Animal Husbandry Building

W. T. CRANDALL, *Chairman*

1:00– 1:15 **Opening Business Session**

1:15– 4:00 **Dairy Record Keeping**

Report of Dairy Records Committee. J. F. KENDRICK. *Bureau of Dairy Industry, U.S.D.A.*

Canadian Registration and Production Policies. G. E. Raithby, *Ontario Agricultural College.*

The Ohio Dairy Service Plan. R. R. STARBUCK, *Ohio State University.*

Estimating the Yield of Pasturage on Farms (a) The Method; (b) Use in Dairy Herd Improvement. R. E. HODGSON, F. J. ARNOLD, J. B. SHEPHERD, AND R. E. SHEAFFER, *Bureau of Dairy Industry, U.S.D.A. and the University of Maryland, cooperating.*

4:00– 5:00 **Committee Meetings**

Wednesday, June 25

Morning Session

War Memorial Hall

W. T. CRANDALL, *Chairman*

9:00–12:00 **Teaching Methods and Exhibits**

Technicolor, sound Extension Teaching Movie, "The Challenge to New York Dairymen".

Inspection of Exhibits and Explanation of Each Exhibit by Person in Charge.

Report of the Exhibit Committee. L. A. Johnson, *Michigan State College.*

Wednesday, June 25

Afternoon Session

Animal Husbandry Building

W. T. Crandall and D. M. Seath, *Co-chairmen*

1:30– 5:30 **Joint Meeting with the Production Sections**

War Memorial Hall

Report of Dairy Cattle Health Committee. C. G. Bradt, *Cornell University.*

Symposium—Brucellosis in Dairy Cattle.

3:00– 3:30 **Recess**

Report of Dairy Cattle Breeding Committee. E. J. Perry, *Rutgers University.*

Report of Breeds Relations Committee. H. A. Herman, *University of Missouri.*

Symposium with Purebred Dairy Cattle Association participating, Getting the Most Out of Our Nation-Wide Testing Program.

Thursday, June 26

Morning Session

Animal Husbandry Building

W. T. Crandall, *Chairman*

9:00–11:00 **Artificial Insemination and Pen Stabling**

Selection and Repeatability of Sires Used in Artificial Insemination. Ray Albrectsen, *Cornell University.*

The Montana Elevated Cow Stall. J. O. Tretsven, *Montana State College.*

The Uninsulated Pen Barn in Wisconsin. G. R. Barrett, *University of Wisconsin.*

11:00–12:00 **Business Meeting**

Thursday, June 26

Afternoon Session

Animal Husbandry Building

W. T. CRANDALL, *Chairman*

1:00– 2:00 **4-H Club Programs**

Report of 4-H Club Committee. G. W. VERGERONT, *University of Wisconsin.*

A 4-H Dairy Extension Program and Plan of Work. H. A. WILLMAN, *Cornell University.*

2:00– 3:00 **Business Meeting**

3:00– 5:00 **General Business Session**

War Memorial Hall

6:30 **Annual Banquet**

Creelman Hall

JOURNAL OF DAIRY SCIENCE

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THE RELATIONSHIP OF THE PREPARTUM DIET TO THE CAROTENE AND VITAMIN A CONTENT OF BOVINE COLOSTRUM

A. A. SPIELMAN, J. W. THOMAS, J. K. LOOSLI, F. WHITING, C. L. NORTON, AND K. L. TURK

Department of Animal Husbandry, Cornell University, Ithaca

The purpose of the experiments reported in this paper was to determine the relationship of the prepartum diet to the carotene and vitamin A content of bovine colostrum.

The literature revealed scanty information concerning the effect of the feed of the dry cow upon the carotene and vitamin A content of colostrum. Kramer *et al.* (5) reported values of 25 and 28 I.U. of vitamin A per gram of colostrum from two cows on rye pasture and values of 16 and 20 I.U. for two cows receiving winter rations. Henry *et al.* (3) noted that cows on pasture before calving produced colostrum containing more carotene than barn fed cows, but that vitamin A was not affected. Stewart and McCallum (11) were unable to increase the vitamin A content of colostrum by feeding 3 lbs. of carrots or one-seventh of a pint of cod-liver oil per day to cows on winter rations. The paucity of available information and the practical importance of colostrum seemed to warrant further study of this problem.

PLAN OF EXPERIMENT

Twenty-nine Holstein and four Guernsey heifers in the Cornell University dairy herd were used in this experiment. These heifers had been on excellent pasture prior to the experiment and were considered to be in good physical condition. Approximately 60 days before calving they were divided into four dietary groups. The experimental diets were as follows: A low-carotene ration composed of wheat straw and a commercial grain mixture containing 12 per cent of protein; a standard dry-cow fitting ration consisting of the same grain mixture plus good quality hay and corn silage; a carotene-rich ration made by supplementing the standard dry-cow ration with one million I.U. of carotene[1] daily; and a vitamin A–rich ration made by

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[1] Commercial concentrate containing 50,000 I.U. per gram, purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

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adding one million I.U. of vitamin A[2] daily to the standard dry-cow ration. Periodic determinations of the potency of the carotene concentrate were made by measuring the absorption of a petroleum ether extract in an Evelyn photoelectric colorimeter at 440 mμ. Vitamin A potency of the shark-liver oil was determined by saponifying a weighed amount of the oil, dissolving the non-saponifiable portion in chloroform, and measuring the Carr-Price reaction in an Evelyn photoelectric colorimeter. The carotene content of the hay (U. S. No. 1 timothy-clover mixed) was 11.6 μg. per gram, and the corn silage contained 5 μg. of carotene per gram on the fresh basis as determined by a slight modification of the chromatographic method proposed by Charkey and Wilgus (2). Feed intakes were not recorded. The experimental rations were fed until the cows calved, after which a regular milking herd ration was fed.

Samples of colostrum were obtained on the first, third, and seventh days following parturition. The first sample, drawn shortly after parturition and before the calf had nursed, was obtained by pooling a pint of colostrum hand-milked from each of the four quarters of the udder. This sample was taken to represent the first colostrum as obtained by the calf following birth. After the first colostrum sample was taken, the cows were machine milked twice daily. All calves were removed from their dams at the end of the second day. The third and seventh day samples were taken from the sixth and fourteenth milkings postpartum. Carotene and vitamin A determinations were made according to the method of Boyer *et al.* (1).

RESULTS

The mean values with their standard error as well as the individual values in each dietary group are given in tables 1 and 2. The significance of mean differences was determined by a *t* test. *P* values of 0.05 arbitrarily are considered as significant, and *P* values of 0.01 are considered highly significant (6).

Carotene

The data presented in table 1 show the relationship of the prepartum diet to the carotene content of the colostrum. Carotene concentration is expressed per unit of volume and per gram of butterfat.

Pre-nursing sample. The difference between the means of the low-carotene group and the standard group is not significant, but the means of the carotene supplemented and the vitamin A supplemented groups are significantly higher when the results are expressed per gram of butterfat. The daily addition of one million I.U. of carotene to the standard fitting ration did not increase significantly the carotene content per 100 ml. of colostrum over that of the standard group. On the other hand, the mean value per

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[2] Shark-liver oil, 41,000 I.U. per gram, generously supplied by National Oil Products Co., Paterson, N. J.

TABLE 1

The effect of the prepartum ration on the carotene content of colostrum

Cow no.	µg. per 100 ml.			µg. per gram butterfat		
	Pre-nursing	Third day	Seventh day	Pre-nursing	Third day	Seventh day
			Low-carotene ration			
R-26	142	21		22.9	14.0	
3	38	21	22	10.8	4.2	3.2
R-86	65	24	29	12.3	4.0	5.2
8	130	51		9.5	5.8	
12	47	54		10.8	6.1	
13*	187*	102*	29*	64.7*	20.6*	8.0*
17	45	19	15	14.5	4.4	2.9
R-95	169	12	5	18.2	4.5	1.0
24	42	28	5	11.3	2.6	0.9
Mean ± S.E.	85 ± 19	29 ± 5	15 ± 5	13.8 ± 1.6	5.7 ± 1.2	2.6 ± 0.8
			Standard dry-cow ration			
1	208	50	21	35.4	9.6	3.9
7	32	61	19	21.4	9.2	3.7
14	170	57	19	22.9	8.1	2.5
R-74	121	40	13	33.5	9.2	3.4
23	70	27	25	15.0	6.5	3.1
34	38	58	38	23.0	8.9	9.2
Mean ± S.E.	107 ± 29	49 ± 4	23 ± 4	25.2 ± 3.1	8.6 ± 0.0	4.3 ± 1.0
			Carotene-supplemented ration			
4	195	123	44	23.6	16.5	9.7
5	187	66	20	31.8	7.5	2.8
9	136	52	18	47.1	9.5	1.9
10	111	63	18	41.4	8.4	3.1
18	46	48	31	44.6	11.6	3.2
29*	198*	86*	41*	68.5*	16.0*	7.9*
30*	790*	127*	117*	173.9*	30.0*	19.5*
31	124	47	11	30.0	5.7	1.8
32	86	96	26	21.9	12.2	3.2
33	140	102	31	48.6	18.6	5.2
Mean ± S.E.	128 ± 17	75 ± 10	25 ± 4	36.1 ± 3.7	11.3 ± 1.3	3.9 ± 1.5
			Vitamin A-supplemented ration			
2	56	105	26	40.2	12.7	6.2
R-64	100	61	15	28.5	11.4	3.1
6	72	38	26	24.1	6.3	3.1
16	400	25	1	61.5	3.5	1.7
20*	111*	80*	34*	71.7*	16.8*	5.4*
22	56		17	18.1		4.4
26	132	20	12	13.9	2.7	2.2
27	57	28	17	14.5	5.7	1.6
Mean ± S.E.	125 ± 32	46 ± 13	16 ± 0.04	28.7 ± 4.1	7.1 ± 1.6	3.2 ± 0.6

* Guernsey not included in mean.

gram of butterfat of the carotene-supplemented group is significantly higher (0.01 level) than that of the other groups.

Third-day sample. The increase in carotene content per unit of volume and per gram of butterfat of the carotene-supplemented group over that of the other groups is highly significant. In contrast to the pre-nursing sample, the mean value of the low-carotene group is significantly lower than that of the standard group.

TABLE 2

The effect of the prepartum ration on the vitamin A content of colostrum

Cow no.	µg. per 100 ml.			µg. per gram butterfat		
	Pre-nursing	Third day	Seventh day	Pre-nursing	Third day	Seventh day
			Low-carotene ration			
R–26	220	32		35.5	21.4	..
3	180	90	66	51.3	18.2	9.7
R–86	320	116	36	60.8	19.4	6.5
8	694	205		51.0	23.4	
12	231	214		53.3	24.4	
13*	86*	174*	11*	29.7*	35.1*	3.0*
17	172	64	47	55.6	14.7	9.2
R–95	188	62	45	19.6	23.1	9.6
24	216	150	24	58.1	14.0	4.3
Mean ± S.E.	278 ± 58	117 ± 23	44 ± 6	48.2 ± 4.5	19.8 ± 1.3	7.9 ± 1.1
			Standard dry-cow ration			
1	418	88	29	71.1	16.9	5.4
7	182	97	12	121.6	14.7	2.3
14	735	202	70	98.9	28.8	9.4
R–74	160	71	33	44.3	16.4	8.6
23	626	117	98	134.8	28.3	11.9
34	124	163	84	75.9	25.0	20.4
Mean ± S.E.	374 ± 107	123 ± 21	54 ± 14	91.1 ± 13.8	21.7 ± 2.6	9.7 ± 2.5
			Carotene-supplemented ration			
4	757	201	92	91.7	26.1	20.2
5	608	192	39	103.4	21.9	5.4
9	318	95	50	100.5	17.4	5.4
10	229	182	54	85.4	24.2	9.3
18	55	78	225	53.3	18.9	23.4
29*	335*	103*	44*	115.9*	19.2*	8.5*
30*	397*	57*	24*	87.4*	13.5*	4.0*
31	226	87	17	54.7	10.5	2.7
32	142	169	65	19.6	21.6	8.1
33	89	93	37	30.8	17.0	6.8
Mean ± S.E.	303 ± 87	137 ± 19	72 ± 23	67.4 ± 11.3	19.7 ± 1.9	10.2 ± 3.2
			Vitamin A-supplemented ration			
2	199	896	98	142.8	108.5	23.4
R–64	239	216	62	68.1	40.3	12.8
6	429	323	43	143.4	54.0	5.1
16	5760†	249	67	885.9†	35.5	11.5
20*	309*	358*	101*	199.6*	75.4*	16.0*
22	962	.....	79	310.7		20.7
26	1750	181	64	184.3	24.3	11.9
27	540	396	139	137.7	80.8	13.4
Mean ± S.E.	687 ± 210 1411‡	377 ± 108	79 ± 11	164.5 ± 32.9 267.5‡	57.2 ± 12.9	14.1 ± 2.3

* Guernsey, not included in mean.
† Unusually high value not included in mean.
‡ Including value †.

Seventh-day sample. Significant differences in the mean values of these samples are found only between the low-carotene group and the standard and carotene-supplemented groups. There is no difference per gram of butterfat.

Vitamin A

The effect of the prepartum ration on the vitamin A content of colostrum is shown in table 2.

Pre-nursing sample. There are no significant differences in the mean values of the low-carotene, the standard, and the carotene-supplemented groups when the results are expressed on a volumetric basis. On the other hand, per gram of butterfat, the difference between the low-carotene group and the standard and carotene-supplemented groups is highly significant. Colostrum from the cows receiving the vitamin A supplement contained 409, 313, and 384 μg. more vitamin A per 100 ml. and 116, 73, and 97 μg. more per gram of butterfat than that from the low-carotene, the standard, and the carotene-supplemented cows, respectively. These differences are highly significant. No explanation is apparent for the extremely high vitamin A value of the pre-nursing sample from cow no. 16.

TABLE 3

The relationship of the diet of the dry cow to the vitamin A potency of colostrum

Ration	Pre-nursing	Third day	Seventh day
	*I.U./100 ml.**	*I.U./100 ml.**	*I.U./100 ml.**
Low-carotene ration	1245	516	201
Standard dry-cow ration	1674	574	254
Carotene-supplemented ration	1425	773	330
Vitamin A-supplemented ration	5850	1584	343

* Assuming 0.6 μg. carotene = 1 I.U.
Assuming 0.25 μg. vitamin A = 1 I.U.

Third-day sample. There is no apparent difference between the low-carotene, the standard, and the carotene-supplemented groups. The third-day sample of colostrum from the cows receiving the vitamin A supplement contained significantly more vitamin A than that from the other groups.

Seventh-day sample. Seven days after calving little difference existed between the vitamin A content of the colostrum from the cows receiving the low-carotene, standard, and carotene-supplemented rations. However, the effects of vitamin A supplementation still were evident, as indicated by the significant increase per gram of butterfat of the vitamin A–supplemented group over the other groups.

The total vitamin A potencies of the colostrum samples, expressed as I.U. per 100 ml., are given in table 3. These results are expressed on a volumetric basis as being representative of the vitamin A intake of the newborn calf. Colostrum from the vitamin A-supplemented cows contained 5,850 I.U. as compared to 1,245 I.U. for the low-carotene group, 1,674 I.U. for the standard group, and 1,425 I.U. for the carotene-supplemented group.. Effects of the vitamin A supplementation still were evident on the third day, this colostrum being approximately three times more potent in vitamin A than that from the low-carotene or standard group.

It was thought that the form of vitamin A present in the diet might be a factor in the mammary transmission of vitamin A. The data shown in table 4 indicate that the vitamin A present in colostrum is entirely of the ester form regardless of the prepartum ration. The analytical method used was essentially that described by Kascher and Baxter (4) for separating the ester and alcohol forms of vitamin A.

TABLE 4

The relationship of prepartum diet to the form of vitamin A in colostrum

Ration	Breed	Carotenoids	Vitamin A	
			Alcohol	Ester
		µg./100 ml.	*µg./100 ml.*	*µg./100 ml.*
Standard dry-cow	H	191	0	335
Carotene (alfalfa leaf meal)–supplemented	G	440	0	163
Vitamin A (ester form)–supplemented	G	724	0	1307
Vitamin A (ester form)–supplemented	H	136	2	213
Vitamin A (alcohol form)–supplemented	H	132	0	325
Vitamin A (alcohol form)–supplemented	H	158	0	251
Vitamin A (alcohol form)–supplemented	H	172	0	558

The plasma carotene and vitamin A values of the experimental cows 60 and 18 days before parturition have been reported (10). Using these values, highly significant positive correlations of 0.509 ± 0.14 and 0.523 ± 0.139 were found between the plasma carotene and vitamin A 18 days before calving and the carotene and vitamin A content of the pre-nursing samples of colostrum.

DISCUSSION

The value of colostrum in combating scours and allied diseases of newborn calves' was demonstrated by Smith and Little (9). Later workers (8, 11) have associated the protective characteristics of colostrum with its high vitamin A content. The data presented here show that the prepartum diet of the bovine may influence markedly the vitamin A activity of colostrum.

Large variations in the carotene and vitamin A levels were observed among these samples of colostrum. Undoubtedly sampling procedure was a factor. However, it is not known whether the butterfat content and the associated fat-soluble vitamins of colostrum vary with the completeness of milking, as is the case with normal milk. By expressing the carotene and vitamin A content on a per gram of butterfat basis, the variations due to sampling should be minimized, since it seems unlikely that the first drawn sample should contain more or less of the fat soluble vitamins per gram of butterfat than the last drawn sample. Further study of this problem is needed.

The second finding in these studies is the lack of increase of vitamin A in the colostrum when one million I.U. of carotene was added daily to the standard dry-cow ration. These results are an interesting corollary to the relatively poor fetal storage of vitamin A in the newborn calves from these cows as previously reported (10). Specific differences in the efficiency of conversion of carotene to vitamin A, the stability of carotene in the digestive tract, and the action of vitamin E in conserving carotene and vitamin A may well be contributing factors.

Although it has been shown that the prepartum diet and the concentration of carotene and vitamin A in the blood stream prior to the decline at parturition influences the level in the colostrum, the processes involved largely are a matter of speculation. Liver reserves of carotene and vitamin A probably play a part and may explain the relatively high colostral carotene and vitamin A of the low-carotene group, since these heifers were on excellent pasture prior to the experimental period. The accumulation of milk in the udder prior to parturition and the observation of Petersen and Rigor (7) that milk left in the udder for several days assumes the composition of colostrum may indicate a simple storage phenomenon.

The question warrants further study as to whether or not supplementing the dry-cow ration with extra vitamin A will result in superior performance by the newborn calf or the cow.

SUMMARY

A study has been made of the relationship of the prepartum diet to the vitamin A and carotene content of bovine colostrum. Four different rations were fed to 29 Holstein and 4 Guernsey heifers during the last 60 days of their gestation periods. The rations were a low-carotene ration of wheat straw and a concentrate mixture; a standard dry-cow ration of concentrate, mixed hay and corn silage; the standard ration supplemented with one million I.U. of carotene daily; and the standard ration supplemented with one million I.U. of vitamin A daily.

Colostrum from cows receiving the low-carotene ration contained significantly less vitamin A per gram of butterfat than did colostrum from cows receiving the standard dry-cow ration.

The carotene content per gram of butterfat in the colostrum from the carotene-supplemented cows was significantly higher than that from the other groups, although the vitamin A content was not increased.

Colostrum from cows receiving the vitamin A supplement contained an average of 687 μg. per 100 ml. or 164.5 μg. per gram of butterfat, while the

colostrum from the standard dry-cow ration group contained only 374 µg. per 100 ml. or 91 µg. per gram of butterfat, showing that the vitamin A content of colostrum may be influenced by the prepartum diet.

Regardless of the form of vitamin A in the ration, the ester form of vitamin A predominated in the colostrum.

Highly significant positive correlations were found between the plasma carotene and vitamin A of the cows 18 days before calving and the carotene and vitamin A content of the colostrum.

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A STUDY OF A CREAM SEPARATOR BOWL WHICH IS CLEANED BY CENTRIFUGAL FLUSHING

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In 1938 the W. and J. Whitehead, Ltd., in Laisterdyke, England, sent a centrifugal cream separator to the Illinois Agricultural Experiment Station. The bowl of this machine was designed to provide cleaning and drying by centrifugal force.[1] In 1945, another Whitehead separator was received by the Experiment Station. Both machines were investigated under various conditions of operation.

PRELIMINARY EXPERIMENTS

Tracy and Tuckey (8) in 1938 conducted a number of experiments with the English machine. The purpose of these early experiments was to study the automatic cleaning principle of this separator and ascertain its practical usefulness under farm conditions. The machine was used on two different farms near the Illinois Agricultural Experiment Station for periods of 5 to 7 days. Its operation also was studied in the dairy laboratories of the Experiment Station and results compared with those of a well-known American separator. The bacterial quality of the cream and skim milk was determined. The results of 24 separations, involving 12 comparisons, indicated that the English machine compared favorably with the American machine when both were operated under comparable conditions. This preliminary investigation indicated that the English separator was cleaned easily, that it was practical, that dairymen were interested in its development, and that the two farmers who used the separator were pleased with its performance. The separator was returned to England and World War II delayed further developments.

METHODS

In general, the washing procedure after each separation was as follows:

1. One pint of water at 100° F. was passed through the machine.
2. Next, 20 lbs. of water containing approximately 0.1 per cent of a

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[1] The bowl has a specially constructed water distributor which allows cleaning solutions to flow from the supply tank to all parts of the bowl while it is in motion. In the shell of the bowl there are three discharge ports, equal distance apart, and each one is closed by a valve which is held in place by a steel spring. The tension in the steel spring is overcome by the centrifugal force so that the ports are tightly closed when the bowl revolves at its normal speed. When the speed slackens to one-half that of normal, the valves open and cleaning solutions can be forced through with turbulent cleaning action.

detergent[2] was flushed through the machine. This flushing was accomplished by first allowing the bowl to decrease in speed, which permitted the port valves to open; then about one-fourth of the water was allowed to run through. The speed of the bowl was increased, but not sufficiently to close the ports, and another one-fourth of the water was flushed through the bowl. This was repeated a third and a fourth time until all the water had been used.

3. The speed of the bowl again was increased to force out the last particles of water.

4. The supply tank and spout assembly were brushed after each separation (beginning with separation 37), using the detergent solution which previously had been flushed through the bowl; and finally rinsed with water at about 100° F.

5. Before separating each lot of milk, 25 lbs. of water at 165–170° F. was flushed through from the supply tank in about four equal portions. Samples of the whole milk, cream, and skim milk were taken with sterile pipettes, placed in sterile tubes, and cooled to about 35° F. with iced water. Plate counts for bacteria were made in duplicate, using tryptone-glucose-extract agar. The plates were incubated at 37° C., for 48 hours. Mold and yeast counts were made in duplicate on potato-dextrose-agar, acidified to pH 3.5 with tartaric acid. The plates were incubated 4 days at room temperature.

Fat tests were made by the Babcock method on the milk and cream and by the American Association method (3) on the skim milk in trials 2, 3, 4, and 5.

EXPERIMENTAL

In July, 1945, a new model of the English separator, slightly different in construction from the first one, was sent to the Experiment Station and subjected to more detailed investigation. In construction, the shell of the bowl and the water distributor were made of aluminum, while the cream screw and valve springs were made of brass and steel, respectively. The supply tank, spout assembly, and discs were not heavily tinned because of the existing tin shortage.

Testing the centrifugal flushing principle. The inventor had claimed that this machine could be cleaned by centrifugal flushing and that it was unnecessary to disassemble the separator for cleaning except at infrequent intervals. The objective in this series of separations was to operate the machine each morning and evening over a period of 2 weeks without disassembling it and to determine the effectiveness of centrifugal flushing by measuring the quality of the cream and skim milk with the plate count method. The results are given in table 1. The machine was operated without diassembling it through separation 36. The spout assembly was inspected and sour and putrid cream was found in the cream spout. Evidently the force of the washing solution from the bowl was not sufficient to remove adhering cream. Beginning with separation 37, the supply tank and spout assembly were washed by brushing each morning and evening with the water that previously had been flushed through the bowl. Including separation 38 and through the remainder of this trial, with the exception of separation 43, the plate counts of the cream do not reveal any significant contamination from one separation to the next. The only cream samples that revealed any yeasts and molds were those from separation 50, which showed 1 yeast and 1 mold colony; separation 51, which showed 1 yeast and

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[2] This detergent is called "Vel" and is the sodium salt of a sulfated monoglyceride. The coco-glyceride is chiefly used. The sodium sulfo-monoglyceride constitutes about 30–55 per cent on a dry basis; moisture 1–2 per cent; tetrasodium pyrophosphate 8–9 per cent, and the balance is mostly sodium sulfate, resulting from the reaction in obtaining the sulfated monoglyceride salt. This detergent was used in all trials and the authors are indebted to the Palmolive-Colgate-Peet Company for the supply used in the experiments reported in this paper.

TABLE 1

The effect on the bacterial plate counts of the cream and the skim milk of operating the English machine for 28 separations (Trial 1)

aration no.	Time	Counts per ml. of:		
		Milk	Cream	Skim milk
25	a.m.	37,000	94,500	65,000
26	p.m.	37,100	96,000	13,700
27	a.m.	7,900	188,000	10,400
28	p.m.	8,600	146,000	7,800
29	a.m.	7,000	65,000	54,000
30	p.m.	4,200	1,630,000	*
31	a.m.	13,800	251,000	6,400
32	p.m.	7,600	1,700,000	14,500
33	a.m.	7,500	35,000	10,400
34	p.m.	8,000	1,250,000	8,500
35	a.m.	2,600	50,000	4,700
36	p.m.	2,600	1,300,000	257,000
37	a.m.	240,000	115,000	110,000
38	p.m.	215,000	174,000	130,000
39	a.m.	4,800	2,100	3,900
40	p.m.	6,200	2,000	32,000
41	a.m.	7,300	1,000	2,000
42	p.m.	9,900	26,000	4,900
43	a.m.	7,400	151,000	76,000
44	p.m.	7,600	10,000	9,700
45	a.m.	3,900	64,000	3,900
46	p.m.	5,500	9,800	47,000
47	a.m.	2,900	6,400	5,900
48	p.m.	3,600	4,900	7,500
49	a.m.	7,000	4,900	7,900
50	p.m.	7,000	10,000	9,700
51	a.m.	471,000	250,000	321,000
52	p.m.	449,000	414,000	806,000

* Sample lost.

9 mold colonies; and separation 52, which showed 17 mold colonies per ml. The plate counts of the milk separated each morning and evening agreed closely, as expected, since the milk was obtained from the same lot.

The bowl was taken apart after separation 52. The lock ring had a bad odor as the result of milk solids which had accumulated in the threads, for it had not been tightened sufficiently at the beginning of the trial. The shell of the bowl was clean, but the dividing disc had some milk solids at the points opposite the holes in the discs. The top disc had a slight deposit of milk solids around each hole, and the amount of this deposit became progressively greater from the top to the bottom disc. The lower surface of the milk distributor had some adsorbed milk solids.

The inventor had claimed that the centrifugal flushing action from the bowl also would be sufficient to clean the spout assembly. However, this series of separations proved conclusively that it is necessary to wash the supply tank and spout assembly after each separation. This was done in all the remaining trials.

TABLE 2

The effect on the bacterial plate counts of the cream and skim milk of operating the English machine for 14 separations without disassembling the bowl, using a combination of detergents (Trial 2)

Separation no.	Time	Counts per ml. of:		
		Milk	Cream	Skim milk
53	a.m.	9,500	4,200	16,000
54	p.m.	13,000	3,500	18,000
55	a.m.	8,300	5,600	11,000
56	p.m.	9,800	7,600	20,000
57	a.m.	20,000	4,800	25,000
58	p.m.	260,000	8,000	40,000
59	a.m.	300,000	15,000	10,000
60	p.m.	15,000	4,000	*2,600,000
61	a.m.	8,000	1,400	5,500
62	p.m.	4,200	1,400	4,800
63	a.m.	11,000	*4,500	5,800
64	p.m.	10,500	*14,000	10,000
65	a.m.	120,000	*75,000	150,000
66	p.m.	15,000	50,000	332,000

* Spore-forming bacteria were present.

In a second trial, the detergent "Vel" was combined equally by weight with another detergent and the mixture used in a concentration of about 0.1 per cent. The washing procedure was the same as that used in the previous experiment. The results are recorded in table 2. The machine was disassembled at the termination of the trial, and the underneath surfaces of all the discs had an adsorbed layer of white material which was, in all probability, a precipitate from the combined detergents. There was no separator slime on the inner wall of the bowl, but small amounts of solid materials were deposited near two of the discharge ports.

Washing the bowl without a detergent. A third trial was conducted to determine if the bowl could be washed without a detergent. Following the flushing of the bowl with the hot water, a pint of cold water was run through the machine to cool it so that milk would not adhere to the metal. The supply tank and spout assembly were washed as in previous trials, with the detergent solution. The milk separated each morning and evening was from the same lot.

The results in table 3 do not reveal any great increases in plate counts of the cream that could be attributed to contamination in the bowl from the preceding separation. The plate counts for bacteria in the cream and skim milk obtained from the duplicate lots of milk that were separated each day are in close agreement.

TABLE 3

The plate counts of the cream and skim milk obtained with the English machine when no detergent was used to wash the bowl during 14 separations (Trial 3)

Separation no.	Time	Counts per ml. of:				
		Milk	Cream			Skim milk
		Bacteria	Bacteria	Yeasts	Molds	Bacteria
67	a.m.	49,000	42,000		5	96,000
68	p.m.	63,000	36,000		8	114,000
69	a.m.	142,000	106,000	1	16	170,000
70	p.m.	180,000	93,000		11	210,000
71	a.m.	10,000	4,200	2	6	14,000
72	p.m.	11,000	5,300	4	7	14,000
73	a.m.	4,500	4,100	2	4	6,600
74	p.m.	6,100	2,300		3	6,800
75	a.m.	71,000	4,200	1	5	86,000
76	p.m.	70,000	6,600		6	87,000
77	a.m.	2,800	9,200		9	51,000
78	p.m.	36,000	11,000		7	47,000
79	a.m.	230,000	129,000	1	2	389,000
80	p.m.	276,000	142,000		3	328,000

Examination of the various parts of the bowl revealed a very slight deposit of milk solids near the holes on the lower side of the first disc. This formation became progressively greater on each disc from top to bottom, whereas the upper surface of every disc was free from milkstone. The base of the bowl spindle was covered with a thin film of white material which was lightly adsorbed. The outer surface of the binding ring had a layer of milkstone, but there was no other deposit on the outside of the bowl.

Comparing the English and the American separators. A fourth trial was conducted to compare the quality of the cream and skim milk obtained by separating duplicate lots of milk with the English and the American separators. The American machine was washed after each separation by the rapid method suggested by Rudnick (7) except that 25 lbs. of water at

165–170° F. was passed through it immediately before each separation. The procedure for the English separator was the same as that outlined. Two 10-gallon cans of milk were obtained from the University dairy. The milk from one can was mixed well and divided equally each morning; one half of it was separated with the American and the other half with the English machine. The same procedure was repeated in the evening with the other can of milk, which had been held at 35–40° F. The results are summarized in table 4. The plate counts of the cream from separations 82 and 106 with the English machine are higher than the comparable plate counts from separations 81 and 105 with the American machine. The plate counts for the cream in the other 12 comparisons and those for all the skim milks obtained with both machines agreed closely.

TABLE 4

Comparison of the bacterial plate counts of the cream and skim milk obtained by separating duplicate lots of milk with the American and English machines (Trial 4)

Separation nos.	Time	Counts per ml. of:				
		Milk	Cream		Skim milk	
			American	English	American	English
81,* 82†	a.m.	3,700	3,800	11,000	3,400	5,600
83, 84	p.m.	2,300	1,100	1,500	2,000	2,200
85, 86	a.m.	6,800	3,200	2,000	4,800	5,300
87, 88	p.m.	10,600	2,500	700	7,500	6,800
89, 90	a.m.	2,700	1,200	1,500	2,400	2,900
91, 92	p.m.	900	900	600	2,000	900
93, 94	a.m.	10,400	‡	‡	9,800	11,000
95, 96	p.m.	2,000	600	1,500	2,800	1,900
97, 98	a.m.	1,700	4,200	900	2,600	2,500
99, 100	p.m.	5,500	500	2,400	2,100	2,600
101, 102	a.m.	2,000	400	1,900	2,400	2,900
103, 104	p.m.	1,100	600	800	1,600	1,700
105, 106	a.m.	1,700	500	38,000	1,100	1,000
107, 108	p.m.	3,500	400	4,800	1,600	1,200

* Odd nos. represent separations with the American machine.
† Even nos. represent separations with the English machine.
‡ Samples lost.

The bowl of the English machine was sanitary at the end of the trial except for the three bottom discs, which showed slight traces of dried milk solids. There was not the slightest trace of milk solids on either the inner shell or the outer surface of the bowl. The rapid procedure used to wash the American machine did not remove all milk solids from the lower surfaces of all the discs.

Separating milk of inferior quality. The milk separated with the English machine in the preceding trials was of good quality, as indicated by relatively low plate counts. It was deemed advisable to determine the effect of separating milk of poor quality on the plate counts of the cream and skim milk and on the centrifugal flushing ability of the bowl in the English machine. Two 10-gallon cans of milk were obtained and dumped into a vat, mixed well, and poured back into the same cans. One can of milk was separated immediately and the other was held at 36–40° F. and separated in the evening. The washing procedure was the same as that outlined.

With the exception of separation 112 (table 5) there was little evidence of contamination from one separation to the next, as indicated by the bacterial plate counts of the cream. Except for separations 109 and 110, there is fairly close agreement in the plate counts of the skim milk each morning and evening from the duplicate lots of milk. The milk used in separations 119 and 120 showed evidence of mastitis, as indicated by the presence of dark-colored coagulated material on a sediment disc. The inner shell of the bowl was inspected and found to be clean before proceeding with separation 119. Immediately after completing separation 120 and after washing by the prescribed procedure, dark material was found adsorbed on the inner shell of the bowl. This material had accumulated during separations 119 and 120 and was not removed by the washing procedure. A sediment test was made of the milk used in separations 119 to 122, inclusive, and the disc scored 6.5 and 7, which is indicative of the poor quality of the milk. At the conclusion of the trial the three bottom discs had small amounts of adsorbed milk solids, but the others were bright and clean on the upper and lower surfaces.

TABLE 5

The effect on the plate counts of the cream and skim milk of separating milk of poor quality with the English machine (Trial 5)

Separation no.	Time	Counts per ml. of:				
		Milk	Cream			Skim milk
		Bacteria	Bacteria	Yeasts	Molds	Bacteria
109	a.m.	12,500,000	1,650,000	2	6	2,220,000
110	p.m.	16,000,000	3,100,000			23,000,000
111	a.m.	20,000,000	6,400,000	2	12	21,500,000
112	p.m.	32,500,000	18,400,000	100	2	14,700,000
113	a.m.	1,200,000	1,500,000	130	100	4,700,000
114	p.m.	5,800,000	1,450,000	80	10	4,100,000
115	a.m.	4,200,000	9,300,000	4	7	5,500,000
116	p.m.	4,400,000	7,200,000	4	5	3,900,000
117	a.m.	300,000	250,000	9	2	330,000
118	p.m.	430,000	220,000	4	5	450,000
119	a.m.	1,000,000	460,000	12	7	1,300,000
120	p.m.	1,500,000	420,000	250	3	1,000,000
121	a.m.	5,800,000	8,000,000	12	2	8,200,000
122	p.m.	3,200,000	6,500,000	5	3	8,600,000

In trial 5, the fat content of the milk averaged 4.6 per cent and that of the resulting cream, 57.43 per cent. The cream contained a higher percentage of fat in this trial because the screw on the bowl did not permit a sufficient range of adjustment for milk of high fat content to obtain cream of what might be called normal fat content. In spite of the high fat content of the cream, the bowl was remarkably well cleaned up to and including separation 118, as indicated by the appearance of the inner shell.

Distribution of bacteria in the cream and the skim milk. This study provided data on the distribution of the bacteria in the cream and skim milk. The results from trials 2, 3, 4, and 5 are shown in table 6; trial 1 was excluded because it was preliminary. In 85 per cent of the separations with the English machine, the cream had a lower plate count for bacteria than the skim milk; in 80 per cent of the separations the cream had a lower count than the original milk. Similar results were obtained by Tracy and Tuckey (8). The results in table 4 also show that the cream obtained with the American machine had a lower plate count than the skim milk in 11 of 13 comparisons. These data agree with the results of seven separations reported by Ulvin and Cree (9). The other data reported in the literature do not agree on the distribution of bacteria in cream and skim milk obtained by centrifugal separation. Eckles and Barnes (2) and Anderson (1) reported higher plate counts in cream than in skim milk. The results of Lamson (5) indicate less or only slightly more bacteria in separated cream than in the whole milk, but that the skim milk contained fewer bacteria than the whole milk. Leete (6) in 100 separations did not find much difference in the bacterial counts of cream, skim milk, and whole milk, the average counts being 501,000, 313,000, and 435,000 per ml., respectively.

TABLE 6

Comparative distribution of the plate counts of the milk, cream, and skim milk for 55 separations with the English separator
(Trials 2, 3, 4, and 5)

Plate counts of:	Total	
	No.	%
Cream lower than skim milk	47	85
Cream higher than skim milk	8	15
Cream lower than milk	44	80
Cream higher than milk	11	20

The factors which determine the distribution of bacteria in cream and skim milk obtained by centrifugal separation should be studied in more detail because of the implications in determining bacterial standards for market cream. It is probable that the physical state of the fat globules and their membrane materials, the temperature of the milk, and the types, numbers, and specific gravity of the microorganisms are factors which determine their retention by either the cream or the skim milk.

Skimming efficiency. The skimming efficiency of the English machine was determined in trials 1, 2, 3, 4, and 5. The results assembled in table 7

TABLE 7

The milk fat content of the skim milk from 84 separations with the English machine

% milk fat in skim milk	No. of separations
0.03	1
0.04	2
0.05	8
0.06	11
0.07	20
0.08	14
0.09	11
0.10	6
0.11	2
0.12	3
0.13	1
0.14	0
0.15	3
0.16	0
0.17	0
0.18	0
0.19	0
0.20	2
Mean 0.08	84

indicate an average of 0.08 per cent fat in the skim milk, which compares favorably with results reported in the literature (4) of 0.06 to 0.08 per cent fat in the skim milk for a factory separator. In trial 5, 14 comparisons were made with the American and English machines. While the fat tests of the skim milk were slightly lower for the American machine, the data are insufficient to draw definite conclusions.

Time required to wash the English separator. Washing this separator by centrifugal flushing of the bowl, including brushing and rinsing the supply tank and spout assembly, varied from 3.5 to 5.5 minutes, depending on the experience of the operator. A person accustomed to the procedure can wash this machine after each separation in about 3 minutes and assemble it for operation in about 0.5 minute. When each experimental trial was terminated, the supply tank, spout assembly, and the bowl were disassembled, the discs inspected and washed, and the machine reassembled. The time required for this operation for one person for whom records are available was 17 minutes.

DISCUSSION

The experiments reported indicate that the English separator cannot be cleaned entirely by centrifugal flushing, as originally claimed by the inventor. However, the bowl can be washed easily and quickly without disassembling it; the supply tank and spout assembly must be washed after each separation. The bowl was cleaned remarkably well by the flushing action of the washing solutions, even though it was necessary to use a mild detergent of low alkalinity because of the aluminum bowl and some of its parts and because of the inadequately tinned discs, supply tank, and spout assembly.

The fact that the plate counts of the cream were lower than those of the skim milk in 85 per cent of the separations is of interest, but it is questionable if these results can be attributed to any superiority of the English machine as similar results were obtained in a few trials with the American machine and in a limited number of trials by other investigators (9).

The skimming efficiency of the English machine of 0.08 per cent fat in the skim milk, using the American Association method (3), compares favorably with results reported for a factory separator.

This separator has been demonstrated to be practicable for farm use. Furthermore, the useful life of the bowl will be prolonged because it will have to be disassembled and washed less frequently than present machines.

CONCLUSIONS

1. The bowl of the English separator can be washed properly by centrifugal flushing with two separations daily over a period of 1 to 2 weeks.
2. In the great majority of cases, there was no significant contamination from one separation to the next, as indicated by the plate counts of the cream and of the skim milk.
3. Presence of 0.08 per cent fat in the skim milk from the English separator as determined by the American Association method compares favorably with results obtained with a factory separator.
4. The plate counts of the cream were lower than the skim milk in 85 per cent of the cases and lower than the milk in 80 per cent of the cases.

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THE EFFECT OF SULFANILAMIDE IN THE DILUENT UPON FERTILITY OF BULL SEMEN

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Since 1939, when the possible effects of bacteria on the results of metabolic studies with spermatozoa or on the results of artificial insemination were first discussed by Salisbury *et al.* (9), this laboratory has been engaged in seeking a solution to that problem (3, 4, 5, 8). Recently, Knodt and Salisbury (5) have shown that 300 mg. of sulfanilamide added per 100 ml. of yolk-citrate diluent resulted in significant improvements in the livability of bull spermatozoa stored in this diluent. The sulfanilamide effectively controlled most of the bacteria normally found in semen and also depressed carbohydrate and oxygen utilization, but it increased the accumulation of lactic acid. Whether or not the stimulation of the spermatozoa to greater livability was due to the control of bacteria or to the effect on their metabolism was not proved.

The present paper deals with the fertility of bull spermatozoa when diluted with yolk-citrate to which sulfanilamide was added at the rate of 300 mg. per 100 ml.

EXPERIMENTAL

Three different experiments were conducted to determine the effect of sulfanilamide on fertility. Each was planned so that the diluent treatments were effectively randomized among the samples of semen collected and used from each experimental bull. The bulls used were those owned by the New York Artificial Breeders' Cooperative, Inc., and inseminations were made by the regularly employed inseminators on cows owned by the members.

The first experiment. The first study was conducted in June, 1945. It was designed to compare the regular yolk-citrate diluent with a yolk-citrate-sulfanilamide diluent containing 300 mg. of added sulfanilamide per 100 ml. and, also, a yolk-citrate-glucose diluent containing 540 mg. of added glucose per 100 ml. The latter diluent was used to determine the effect of added glucose upon fertility, for it had been shown earlier (10) that glucose effectively increased livability of and lactic acid production by bull spermatozoa.

The citrate buffer containing 3.6 g. of sodium citrate (dihydrate) per 100 ml. was prepared with water distilled in glass and autoclaved for 20 minutes at 15 lbs. pressure. Glucose was made up in approximately

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isotonic solutions (5.4 per cent) and sterilized in the same way as the citrate solutions. The yolk-citrate-glucose diluent was prepared by adding 20 ml. of isotonic glucose solution to 80 ml. of citrate buffer, and this mixture was added to 100 ml. of yolk to give a level of 540 mg. of glucose per 100 ml. Various multiples of these proportions were utilized, depending upon the total need for diluent.

The citrate-sulfanilamide solution was prepared by heating water distilled in glass to approximately 80° C. and then dissolving in it the desired amount of sulfanilamide and citrate to give a final concentration of 3.6 g. sodium citrate (dihydrate) and 600 mg. of sulfanilamide per 100 ml. This solution was made up to a predetermined volume before autoclaving at 15 lbs. pressure for 20 minutes. Equal volumes of yolk and citrate-sulfanilamide solution then were mixed for the final diluent. In the first investigation, all diluents were made up before the investigation started and no precautions were taken to prevent direct light from falling on the citrate-sulfanilamide diluent during the 26 days which were required to complete the collections.

Ten bulls were used, 5 Holsteins and 5 Guernseys. Three collections at intervals of 1 week to 10 days were taken from each bull, and the diluent to be used for any particular collection for any one bull was assigned at random. Thus each bull was a block and the design was that of a randomized block.

All semen was diluted so that each milliliter of diluted semen contained 30 million spermatozoa. This was done in order not to have dilution rates as a confounding factor. Later (7) it was found that this precaution was unnecessary within the limits of dilution used and it was not continued in the two subsequent experiments. Semen was shipped to 47 member units of the Cooperative.

Table 1 shows the data for the average quality characteristics of the semen used with each diluent. Statistical analysis of the data showed no significant differences between the semen samples used for each treatment. As can be seen, additions of glucose apparently increased methylene blue reduction time, but this difference was not statistically significant. Also, the results of the inseminations are shown in table 1. It is obvious that no difference in fertility was found between the yolk-citrate and the yolk-citrate-sulfanilamide diluents in this experiment.

In view of our more recent investigations, the authors are of the opinion that as a consequence of storing in clear bottles on the laboratory desk, the sulfanilamide in the alkaline citrate (pH about 7.45) was oxidized by the action of light, as evidenced by a slight browning, and thereby lost its effectiveness. This view is supported by data from a later experiment in which a limited number of ejaculates were treated with freshly prepared citrate-sulfanilamide as compared to the citrate-sulfanilamide diluent which

TABLE 1

Average semen quality characteristics and fertility results in the first experiment

Semen character	Diluent used		
	Yolk-citrate	Yolk-citrate-glucose	Yolk-citrate-sulfanilamide
Concentration 1,000's/mm.^{3}	1,232	1,280	1,190
Motility, %	80.0	79.0	79.0
Methylene blue reduction time, min.	3.88	4.68	4.08
Fertility			
Services, no.	786	720	767
5-mo. N.R.,* no.	377	352	368
5-mo. N.R., %	48.0	48.9	48.0

* N.R. = non-returns to service.

had been exposed to light. The action of light brought about changes in the sulfanilamide solution so that it failed to stimulate spermatozoan livability.

The addition of glucose to the citrate resulted in a slightly smaller proportion of returns to service during the 5 months following service, but the difference was not mathematically significant. There was some evidence in the data that the samples to which the glucose was added had been used more days in the field than the other samples, but again this difference was not statistically significant.

The second experiment. The second fertility experiment was conducted in November, 1945. The design was essentially the same as in the first experiment. Collections from ten bulls, 5 Holsteins and 5 Guernseys, were made twice with an interval of 7 to 11 days. To one or the other of these collections the diluent to be used, yolk-citrate or yolk-citrate-sulfanilamide,

TABLE 2

Average semen quality characteristics and fertility results in the second experiment

Semen character	Diluent used	
	Yolk-citrate	Yolk-citrate-sulfanilamide
Concentration 1,000's/mm.^{3}	1,189	1,187
Motility, %	79.5	78.5
Methylene blue reduction time, min.	4.82	4.88
Fertility		
Services, no.	676	765
5-mo. N.R.,* no.	379	476
5-mo. N.R., %	56.1	62.2

* N.R. = non-returns to service.

was assigned at random. In this investigation the two buffers for the diluents were made up and sterilized as described above, but were stored in the dark during the 18 days required to complete the semen collections. The diluted semen was shipped to 66 local units of the Cooperative. Table 2 gives the average semen quality characteristics and the results of the inseminations.

In this experiment the results were strikingly different from those obtained in the first one. An analysis of covariance, using the number of services as the independent variable and 5-month non-returns as the dependent variable, indicated that the 6.1 per cent difference in level of fertility for the diluents was mathematically significant at the 5 per cent level of probability.

The varying results of the first and second experiments suggested that the different fertility levels observed might be due simply to the random variability typical of biological experiments. The number of sulfanilamide-treated semen samples was small, only 10, and the level of odds for the differences in fertility was just barely over the 5 per cent point.

The third experiment. A third experiment was planned to test the matter more thoroughly. It was desired to study not only the effect of sulfanilamide on fertility, but to study certain other questions as well. Work by Gunsalus *et al.* (4) had indicated that first ejaculates contained a higher concentration of bacteria than did second ejaculates collected a few minutes later. Mercier *et al.* (6) found that first ejaculates were more concentrated, but were of lower volume and contained spermatozoa of which a somewhat smaller percentage were motile. As far as was known, no controlled investigation had been conducted to determine whether first ejaculates were less fertile than second ones, though such an opinion was known to be held by some workers in this field. If such opinions were found to be based upon fact, the question arose as to whether sulfanilamide, supposedly by bacteriostatic action, would eliminate the difference between first and second ejaculates.

The third experiment was conducted during June, 1946, and 16 bulls, 12 Holsteins and 4 Guernseys, were used. The bulls within a breed were paired at random. Two collections, spaced on the average 14 days apart, were made from each bull. At each collection two consecutive ejaculates of semen were obtained and used separately. For any one bull at each collection period one or the other of the ejaculates was diluted with the diluent containing 300 mg. of sulfanilamide per 100 ml. At the next collection period either the first or second ejaculate, whichever had not been treated with sulfanilamide the previous time, received it. The design was thus a $2 \times 2 \times 2$ replicated 8 times and is illustrated thus:

Design of the third experiment

	Ejaculate no.	Collection 1	Collection 2
Bull 1	1	Y-C-S*	Y-C†
	2	Y-C	Y-C-S
Bull 2	1	Y-C	Y-C-S
	2	Y-C-S	Y-C

* Y-C-S = Yolk-citrate-sulfanilamide.
† Y-C = Yolk-citrate.

In order to record the identity of each ejaculate, only one of each collection could be shipped to a particular inseminator. Thus, the number of inseminating units to which semen was shipped (76 different circuits) was divided into two approximately equal groups. For example, at one collection one group of circuits received one ejaculate, the second group the other ejaculate. At the next collection the same circuits received the same ejaculate number but the treatment was different. This design involved the willful confounding of the groups of circuits to which the semen was shipped, with a comparison of first and second ejaculates. This fact did not invalidate the test of whether or not first ejaculates responded differently to sulfanilamide than did second ejaculates, for the design was orthogonal in this respect.

The citrate-sulfanilamide buffer was prepared by bringing a measured

TABLE 3

Average semen quality characteristics and fertility results in the third experiment

Semen character	Diluent used			
	Yolk-citrate		Yolk-citrate-sulfanilamide	
	Ejac. 1	*Ejac. 2*	*Ejac. 1*	*Ejac. 2*
Volume, ml.	5.30	5.78	5.38	5.97
Concentration 1,000's/mm.^{3}	1,397	1,178	1,409	1,188
Motility, %	71.9	73.8	70.6	72.2
Methylene blue reduction time, min.	4.2	5.1	4.3	5.3
Fertility				
Services, no.	1,392	1,092	1,149	1,151
5-mo. N.R., no.	821	661	716	761
5-mo. N.R., %	59.0	60.5	62.3	66.1
Combined				
Services, no.	2,484		2,300	
5-mo. N.R.,* no.	1,482		1,477	
5-mo. N.R., %	59.7		64.2	

* N.R. = non-returns to service.

quantity of water distilled in glass to a boil and adding the predetermined quantity of citrate and of sulfanilamide. The solution immediately was removed from the heat source, poured directly into sterile bottles, and stored in the dark.

The data on semen quality and results of insemination are shown in table 3. The data are arranged to give a comparison of first and second ejaculates and to show the effect of diluents on the fertility of the first and the second ejaculates. If one were interested only in a semen-quality comparison of first and second ejaculates before treatment in this study, the proper comparison is between first ejaculates for yolk-citrate and the second ejaculates for yolk-citrate-sulfanilamide. The other consecutive paired ejaculates are the first for yolk-citrate-sulfanilamide and the second for yolk-citrate.

Statistical analysis of the semen quality characteristics showed that the bulls differed significantly only in volume of semen produced. The specification that two consecutive ejaculates which were satisfactory for use be collected at each of two collection periods was responsible for this result. No semen sample was considered acceptable which contained less than 800,000 spermatozoa per mm.^{3}, or which contained less than 60 per cent motile spermatozoa. A total of 20 bulls was sampled for this experiment, 4 of which failed to meet the above specifications.

The spermatozoa of the second ejaculate were significantly more motile than those of the first ejaculate. Highly significant differences were found between first and second ejaculates in concentration and methylene blue reduction time. The first ejaculates were somewhat more concentrated and reduced methylene blue faster than did the second ejaculates. Other differences, as between collection periods, were not of important magnitude and were not mathematically significant.

It should be mentioned that the insemination data were recorded separately for cows being bred for the first time and those which had failed to conceive on first service and were being returned for a second service. The same procedure was followed for the two earlier experiments discussed. In the first two experiments the number of observations was too small to determine whether or not there was a real difference in fertility level for these two groups of cows. In the third, where more observations were made, the difference found was only 1.0 percentage unit, but this small difference was statistically significant. However, the data showed that the addition of sulfanilamide to the diluent increased fertility the same average amount in both groups of cows. In each experiment an analysis of covariance was made on the length of time after collection semen diluted with each diluent was used for insemination. No significant differences were found. For the three experiments combined, 1 per cent of the inseminations were made on the first day (the day of collection), 71 per cent on the second day, 24 per cent on the third, 3 per cent on the fourth, and 1 per cent of all inseminations on the fifth day or later.

As can be seen in table 3 the difference in favor of sulfanilamide in this experiment amounted to a total of 4.5 percentage units. That is, of the 2,300 cows bred with the semen to which sulfanilamide was added, 4.5 per cent more cows apparently conceived than was the case with the 2,484 cows which were bred with the normal yolk-citrate diluent. Statistical analysis of covariance indicated that this difference was greater than was required to show significance at the 1.0 per cent level of probability. Thus, it is concluded that sulfanilamide added to the diluent does increase significantly the probability that a cow will conceive from artificial insemination.

In spite of the fact that no significant differences in semen quality were found between bulls, the differences in fertility among the bulls used was highly significant. However, there was no evidence to indicate that the semen of the several bulls reacted differently to the treatments employed, even though it is known that bacterial contamination varies exceedingly from bull to bull. This fact suggests that the effects of sulfanilamide are largely through the metabolism of semen, and that all normal bull semen would tend to react similarly.

Though first ejaculates tended to be slightly lower in fertility than second ones, an observation bearing out popular belief, the difference observed was not large enough to be mathematically significant when subjected to a test of significance. To test this item effectively a carefully planned and larger experiment should be conducted. The averages given in table 3 suggest that first ejaculates responded less to sulfanilamide treatment that did second ejaculates. However, the sum of squares for error of estimate for the interaction was slightly smaller than the error term, which result forces the authors to conclude that the response to sulfanilamide was not proved different between first and second ejaculates. This observation strengthens the interpretation that the effects of sulfanilamide are largely metabolic ones, for if the effects were due to bacterial control alone one might expect the fertility of the first ejaculates, which contained more bacteria, to be increased more than second ejaculates.

DISCUSSION

It should be mentioned that none of the bulls used in these investigations were known to produce semen containing *Pseudomonas aeruginosa*. This bacterial species is difficult to control and is known to reduce fertility of semen in which it is found as the predominant type (3, 4). Though it was not possible to examine each of the semen samples used in the investigations reported here, the semen of the bulls used had been examined from time to time for bacterial numbers and types, and *Pseudomonas aeruginosa* had not been found.

Reports of the teratogenic effects of sulfonamides when brought into direct contact with certain of the lower animals in the early stages of their development have appeared in the literature (1, 2, 11, 12). While the authors have not examined each calf produced by the sulfanilamide-treated semen, a number of such animals have been examined and no abnormalities observed. Neither have reports of the birth of abnormal calves been received. When one considers the fact that each milliliter would contain only 3.0 mg. of sulfanilamide, such an amount inseminated into the uterus probably would be absorbed quickly and little would find its way to the developing egg.

The practical value of the results of these studies in routine artificial insemination is obvious. However, in the storage of diluents containing sulfanilamide, care must be taken to keep them out of the light. Preferably, the buffer should be made fresh. However, it is difficult to do so and in large operations this may be a nuisance. Sulfanilamide goes into solution slowly. The authors believe that the simplest way to make the diluent is to bring a required volume of properly distilled water to a boil, add the required amounts of both sodium citrate and sulfanilamide, remove the source of heat, and, when the material has been dissolved and thoroughly mixed, pour the buffer thus prepared into sterile, dark bottles and store it in the dark until used. Buffers so prepared have been used for 2 weeks with no observable influence on their effectiveness. How much longer they can be used satisfactorily has not been determined.

SUMMARY

Three separate experiments involving a total of 8,498 inseminations were conducted to determine the effect on fertility of bull semen of adding sulfanilamide to the yolk-citrate diluent at the rate of 300 mg. per 100 ml. In the first experiment no benefit was observed. In the next two investigations, where the citrate buffer containing sulfanilamide was protected from direct light rays, an increase in fertility by use of the sulfanilamide was obtained. This improvement amounted to 6.1 per cent of the cows inseminated in the second experiment and 4.5 per cent in the third.

The sulfanilamide appeared to influence all semen samples in the same direction, for, in the third experiment, no significant interactions were observed between the treatments and either bulls or first and second ejaculates. These results are interpreted as indicating that the beneficial effects of sulfanilamide on fertility largely are metabolic ones, rather than due to bacterial control alone.

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THE EFFECT OF REFRIGERATOR STORAGE ON THE KEEPING QUALITIES OF PASTEURIZED MILK

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Considerable attention has been directed recently to the problem of the keeping quality of milk in the household refrigerator. The economy of alternate-day or 3-day-a-week delivery of milk was amply demonstrated during the war years with the result that most distributors would prefer to retain this practice. On the other hand, some public health officials have questioned the advisability of maintaining this system of milk distribution because of the highly perishable nature of the product. Under the 3-day-a-week system of delivery the product is consumed in from 1 to 4 days after pasteurization. Therefore, to justify such a practice, we must assure the consumer that the product delivered will retain its initial high qualities for at least 4 days.

Several investigators have demonstrated that good quality, commercially processed milk can be held in a household refrigerator for a considerable period of time without impairment to its nutritional qualities or bacteriological safety.

Nicholas and Anderson (8) studied the keeping qualities of regular pasteurized, pasteurized-homogenized, and raw milks under household refrigerator conditions (40° F.). They found that pasteurized milks could be stored at 40° F. for a period of 2 weeks or more before spoilage would occur. Furthermore, milks that were removed from the refrigerator each day, shaken, and allowed to stand at room temperature for 1 hour retained high quality for approximately 10 days. These investigators based their conclusions on the results of standard plate counts, titratable acidity, and flavor observation, but did not take into account the psychrophilic or coliform organisms. Since the standard plate counts at the time of souring of most of the milks used in their studies were very little different from those of the original fresh milk, it would appear that a consideration of psychrophilic organisms would have been helpful in interpreting their data.

Mott and Mayer (7) conducted a study involving the collection of retail milks in Boston. They found that the standard plate count of grades A and B pasteurized milks increased after the samples were stored at 40° F., and had average counts of 1,300,000 and 1,700,000, respectively, after a storage period of 5 days. Twenty-two samples of certified-pasteurized milk had an average count of 770 per milliliter after 5 days' storage at 40° F.

A far more comprehensive study was reported by Dahlberg (3), who obtained samples from six milk plants in the New York metropolitan area

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and stored them at various temperatures. The freshly pasteurized milks had an initial, arithmetic average plate count of about 12,000 colonies per milliliter. Allowing these milks to stand at room temperature for 6 hours had the immediate effect of increasing the plate count slightly and giving more positive coliform tests than were observed with samples not exposed to room temperature. Storage temperatures of 35–40° F. decreased the standard plate count of the milk, while the coliform bacteria remained constant. Samples stored at 45–50° F. showed reduced standard plate counts after 1 day, but considerably higher counts after 4 days. At a storage temperature of 55–60° F. a slight decrease in the standard plate count was observed after 1 day, but thereafter the count increased rapidly. All samples in the group were sour after 4 days. With the exception of this latter group, all samples exhibited good flavor after 7 days' storage. Dahlberg concludes that to insure good keeping quality, milk should not be stored at temperatures above 50° F.

Weber (9) reported an analysis of 10,000 coliform tests collected over a 3-year period for three health departments. These data showed that 21.5 per cent of all pasteurized milks tested were positive to the coliform test during the period of January, February, and March, while 48.0 per cent of the tests were positive during July, August, and September. The higher incidence of coliform-positive milk during the warm months was attributed primarily to a greater degree of contamination of the milk from equipment which is exposed to flies, insects, and air-borne sources.

Dahlberg (4) demonstrated more rapid development of coliform bacteria in refrigerated milk during the summer than during the month of October.

A preliminary report (2) covering certain phases of the present study showed that most commercially pasteurized milks could be held for at least 7 days during the summer months if refrigerator temperatures were held at 45° F. or lower.

The present study was undertaken in order to obtain a more complete picture of the changes that take place during the refrigerator storage of commercially processed market milks, under both summer and winter conditions. It was felt that much of the work reported previously was far too narrow in scope and did not present a complete picture of the bacteriological changes characteristic of stored milk.

METHODS

Two separate studies were undertaken, one under summer conditions (July and August) and the other during the winter (February and March). In each study eight different lots of milk were collected from five different commercial plants immediately after the bottling operation. One-half pint and quart samples of the same milk were collected, and where both homogenized and regular milks were available, a set of each was studied. Samples were iced and transported to the University laboratories, where exposure treatments, bacterial analyses, vitamin analyses, acidity tests, and flavor observations were conducted prior to storage.

As a standard control, one-half pint samples were stored under the cold unit of a large household-type refrigerator. A sufficient number of these samples was stored so that a different bottle could be removed for analysis and observation at each interval of the storage period.

Of the replicate quart samples, one was placed in the refrigerator immediately, a second was allowed to stand at room temperature (summer, 81–89° F.; winter, 74–79° F.) for 1 hour prior to storage, and a third was exposed to room temperature for 2 hours before refrigeration. All quart samples were placed in the refrigerator on the side away from the cold unit. A careful study of temperature variations was made, using recording and maximum-minimum thermometers. The one-half pint samples stored under the cold unit were held at temperatures ranging from 36–42° F. (2.2–5.5° C.) during the summer study and 37–39° F. (2.8–4° C.) during the winter study. The temperature of storage for quart samples held on the side away from the cold unit ranged from 41–45° F. (5–7.2° C.) during the summer study and 39–42° F. (4–5.5° C.) during the winter study. Therefore, the temperature differential between the samples at the two positions of storage was about 3–5° F. during the summer and about 2–3° F. during the winter.

When quart samples were removed for periodic analyses, they were exposed to room temperature for 5 to 7 minutes before being returned to the refrigerator. During this time the temperature of the samples increased about 2–4° F. in the summertime and about 2° F. in winter.

Bacteriological, vitamin, acidity and flavor analyses were made daily for the first 4 days of storage and then at 7 and 10 days. After 10 days, analyses were run every other day until samples showed evidence of acidity change. At this point, samples were examined daily until 0.03 per cent acid had developed. Samples in which 0.03 per cent acid had developed were considered to be "sour," although the flavor of the product was usually quite good and definitely not sour to the taste.

The standard plate count at 37° C. (98.6° F.) (1) was employed for the detection of mesophilic organisms. Dilutions of 1:100 and 1:1000 were employed at the beginning of storage and gradually increased as storage progressed and counts increased. Psychrophilic organisms were similarly determined with standard tryptone-glucose-extract-milk agar plates incubated at 8–10° C. (46.4–50° F.) for 10 days. One milliliter and 0.1 ml. samples were plated for the first few days of storage, after which time high counts necessitated considerable sample dilution.

Coliform organisms were determined on desoxycholate agar plates incubated at 37° C. (98.6° F.) for 24 hours prior to counting; a 5 ml. sample was plated, using one plate with 2 ml. and one with 3 ml. When counts became high, smaller amounts or dilutions were plated.

Acid development was measured by the standard titration technique using phenolphthalein as the indicator and is expressed as lactic acid.

Riboflavin analyses were conducted according to the method of Hand (5), using a Coleman photofluorometer for measuring fluorescence. Ascorbic acid was determined by the conventional titration procedure employing sodium 2,6-dichlorobenzenoneindophenol dye.

RESULTS

Trend of Bacterial Development during Storage

The regular pasteurized milk usually displayed somewhat better keeping qualities than the homogenized product from the same plant. For the first 7 days of storage the differences usually were insignificant, but thereafter the homogenized milks appeared to support the growth of mesophilic and psychrophilic bacteria better than the unhomogenized product. The type of growth and growth curves were very similar. It was considered justifiable to average the counts of both types of similarly treated milk for the first 7 days of storage. Also one plant participating in the study produced only homogenized milk, while another sold only regular unhomogenized milk.

Since most milk is consumed before it is 7 days old, it seemed unnecessary to make a complete tabulation of bacteriological data taken after this time. Therefore, a geometric mean average was tabulated for each class of samples which had been subjected to the same treatment during the same season.

A graphic presentation of bacteriological data is shown in figure 1. It is very evident that all types of bacterial development were more rapid during the summer season. One factor which undoubtedly contributed to this condition was the slightly higher temperatures that prevailed in the refrigerator during the summer months. Another factor which may have influenced this trend was the greater "temperature shock" given quart samples during the warm summer months both before storage and on days when analytical samples were removed. A difference in the type of microflora present in summer and winter milks also might have contributed to this difference.

These data demonstrate the fact that any treatment which allows the milk to warm up will be reflected in the rate of development of all types of bacteria. This is especially evident from the data taken during the summer months. Furthermore, it can be seen that the more extensive the period of warming before storage, the poorer was the bacteriological keeping quality.

For the most part, mesophilic counts went down or remained rather constant for the first few days of storage, after which their rate of development increased according to the degree of exposure to room temperature (samples 2, 3, and 4). In summer milks which were subjected to warming either before storage or on days when samples were analyzed, the mesophilic count did not show any evidence of increasing until after 2 days of storage.

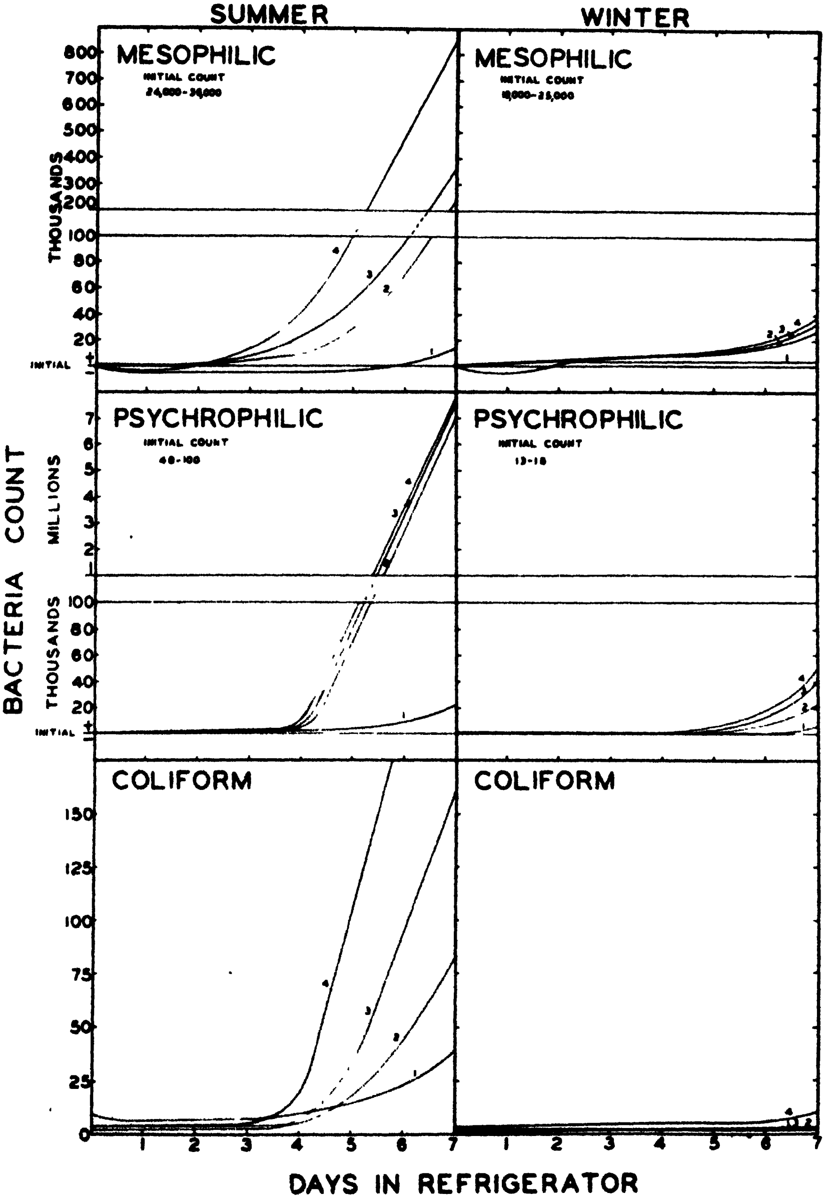

FIG. 1. The effect of refrigerator storage on the micro-flora of commercial market milks (geometric mean averages of all data taken). (1 = one-half pints left in refrigerator continuously; 2 = quarts removed from refrigerator each day for sampling; 3 = quarts allowed to stand out for 1 hour prior to storage, also removed each day for sampling; 4 = quarts allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.)

Undisturbed one-half pint samples (sample 1) showed very little change during 7 days of storage. All samples in the winter study, regardless of treatment, showed very little change during the first 7 days of storage, although the effect of warming before storage was becoming evident in samples 2, 3, and 4 at the end of this period.

The development of the psychrophilic organisms followed a similar pattern, but in the summer series their numbers far exceeded those in the mesophilic class after 6 days of storage. These organisms did not increase appreciably during the first 4 days of storage, but after this time they multiplied at a rather rapid rate. In the winter study the psychrophilic counts did not become significant until after 7 days of storage.

Possibly some mesophilic types may be facultative in the sense that they develop psychrophilic activity after adaptation to prolonged low temperature storage. Support for this view may be found in the fact that mesophilic counts frequently decrease during the first few days of storage. Actually these organisms may be going through a temperature adaptation period which eventually brings them into the active and rapidly growing psychrophilic group. Further support for this possibility is the fact that initial psychrophilic counts of the fresh pasteurized milks in this study usually were very low (0–30). If all growth at low temperatures were due to these few true psychrophiles, probably this growth would develop on a more uniform curve. Since rapid growth of psychrophiles does not occur until after 4 days of storage, probably so-called facultative types may supplement the true psychrophilic population in the final rapid deterioration of the milk.

The development of the coliform organisms followed a pattern similar to that of the mesophiles and psychrophiles. As previously observed by Weber (9), the initial coliform counts in summer milks exceeded those of winter milks. Only two samples were found to be free from coliform bacteria and seven samples showed less than one coliform organism per milliliter during the summer trials. All samples were coliform positive at the conclusion of this series or when 0.03 per cent acid developed. On the other hand, six samples were found to be coliform free and nine had less than one organism per milliliter at the beginning of the winter study. Fourteen samples showed no coliform bacteria at the termination of these trials. In the overall tabulation (fig. 1) the coliform count in winter milk was little changed during the first 7 days of storage.

In evaluating these experimental data in terms of the practical keeping problem in home refrigerators, the conclusion seems justified that good quality commercially processed and distributed milks should maintain desirable bacteriological qualities for at least 4 days when stored at 40° F. Under winter conditions bacterial changes are insignificant from a quality standpoint until after 6 or 7 days of storage. In determining the bacteriological quality of stored milk, the psychrophilic organisms must be taken into ac-

TABLE 1

The comparative micro-flora of freshly pasteurized milk and the same milk after storage and development of 0.03 per cent lactic acid (random samples)

No.	Season of year	Type of bottle	Treatment*	Bacteria counts				Total storage period (days)
				Mesophilic		Psychrophilic		
				Fresh	After storage	Fresh	After storage	
1	Summer	½ pint	A	290,000	38,000	13	177,500,000	21
2	"	½ pint	A	9,000	530,000	8	167,000,000	24
3	"	½ pint	A	61,000	83,000	21	131,400,000	25
4	"	Quart	B	13,600	5,800,000	9	270,000,000	13
5	"	Quart	C	12,200	3,500,000	8	190,000,000	13
6	"	Quart	C	292,000	162,000	26	189,000,000	14
7	"	Quart	D	315,000	221,000	23	178,000,000	13
8	Winter	½ pint	A	14,900	19,600	74	164,000,000	33
9	"	½ pint	A	94,000	213,000	4	248,000,000	35
10	"	Quart	B	18,400	42,000	24	328,000,000	23
11	"	Quart	B	5,000	165,000	1	277,000,000	24
12	"	Quart	C	18,600	21,900	31	315,000,000	23
13	"	Quart	C	67,000	1,730,000	270	150,000,000	12
14	"	Quart	D	128,000	1,650,000	5	260,000,000	20
15	"	Quart	D	39,000	450,000	< 1	201,000,000	17

* A = One-half pint—left in refrigerator continuously; B = Quart—removed from refrigerator each day for sampling; C = Quart—allowed to stand out for 1 hour prior to storage, also removed from refrigerator each day for sampling; D = Quart—allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.

count because of their relatively greater numbers after 5 or 6 days at storage temperatures.

Changes in Micro-Flora during Storage

In analyzing the data taken in both the summer and winter studies and attempting to correlate the initial and final bacterial counts with the relative keeping quality of a product, nothing could be established as an index for a potential trend in bacterial growth. The data presented in table 1 represent a random selection of samples from these studies. These data demonstrate the fact that the initial mesophilic count of the freshly pasteurized milk is not a dependable index of the keeping quality of the product from a bacteriological standpoint. The initial psychrophilic count does not serve as a basis upon which to predict the potential keeping quality of any type of milk, regardless of treatment.

By comparing the data for samples no. 1 and 2 in the summer study and 8 and 9 in the winter study, it can be seen that high count milks (nos. 1 and 9) can have keeping qualities as acceptable as similar milks (nos. 2 and 8) with relatively low initial counts. This should not be taken to imply that the standard plate count is not a useful test for evaluating general milk quality, but it does suggest that its use as an index of potential keeping quality of pasteurized milk is questionable and probably unsound.

Acid Development in Stored Milks

The length of the storage period which elapsed before milks developed 0.03 per cent acid (expressed as lactic acid) naturally varied among the different lots and treatments. One-half pint samples which remained undisturbed in storage did not develop 0.03 per cent lactic acid until 21.8 days (average) of storage in the summer study and 26.6 days (average) in the winter trials. Quart samples which were allowed to stand out at room temperature for 2 hours before storage and were further exposed at periodic sampling periods developed 0.03 per cent lactic acid in 12.4 days (average) in summer and 16.6 days (average) in winter trials.

Most samples examined in these studies showed evidence of lactic acid development while mesophilic counts (standard plate) were still quite low. Some samples developed 0.03 per cent lactic acid while exhibiting a mesophilic count lower than that of the original fresh milk. Nicholas and Anderson (8) have reported "sour" flavor in samples with mesophilic counts which were little different from those of the fresh products.

In the present study the psychrophilic organisms largely are responsible for the development of the lactic acid in milk held in refrigerator storage at 40° F. The data plotted in figure 2 are for three samples, chosen at random from the many which were plotted and compared. A close correlation exists between the growth of psychrophilic bacteria and the development of lactic acid. By comparison, the magnitude of change in the numbers of mesophilic organisms is relatively insignificant. This would suggest that the acid developed, at least in the early stages of deterioration, is a product of the psychrophilic bacteria. Apparently consideration of psychrophiles is of paramount importance in studies of this kind, particularly since acid development is considered to be one of the primary factors in milk spoilage or deterioration.

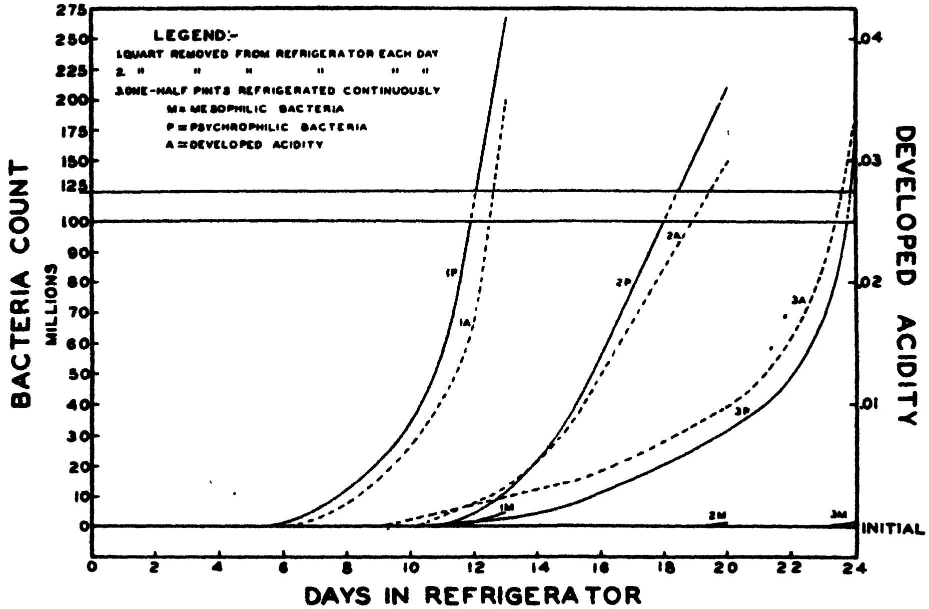

FIG. 2. The relationship between lactic acid development and bacterial growth in milks refrigerated for long periods of time (random samples from different periods during study).

The Effect of Refrigerator Storage on Riboflavin and Ascorbic Acid

Since riboflavin and ascorbic acid are subject to destruction by exposure to light and the latter is subject to natural oxidation on storage, a study of these two vitamins in stored milks appeared desirable.

The data shown in table 2 demonstrate the stability of riboflavin and ascorbic acid in milks stored under household refrigerator conditions. Because of the absence of light in the refrigerators, it is not surprising that riboflavin was stable in samples held continuously under these conditions. Even the quart samples B, C and D, which were exposed to the light of the room at the time of periodic samplings, still retained practically all of their riboflavin throughout the extended storage period. Apparently the normal daylight present in the laboratory was insufficient to cause any great degree of photolysis of this vitamin during these periodic exposures. These results confirmed our previous observations (6) where samples exposed in a bright kitchen for 2 hours lost only a small percentage of riboflavin.

TABLE 2

The effect of refrigerator storage on the stability of riboflavin and ascorbic acid (random samples)

No.	Season of year	Type of bottle	Treatment*	Ascorbic acid mg./l.			Riboflavin mg./l.		Total storage period (days)
				Fresh	After 1 day	After 4 days	Fresh	After storage	
1	Summer	½ pint	A†	7.1	1.6	0.0	1.51	1.52	28
2	"	½ pint	A	8.3	3.2	0.0	1.62	1.61	31
3	"	Quart	B	11.3	9.7	2.0	1.57	1.56	13
4	"	Quart	C†	14.0	13.5	12.1	1.61	1.61	14
5	"	Quart	D	10.1	8.1	2.8	1.55	1.52	12
6	Winter	½ pint	A†	9.1	2.8	0.0	1.62	1.63	25
7	"	½ pint	A†	15.1	12.7	12.0	1.73	1.71	24
8	"	Quart	B	9.1	0.0	0.0	1.73	1.71	15
9	"	Quart	C	6.7	2.0	0.0	1.39	1.37	20
10	"	Quart	D	6.6	1.9	0.0	1.40	1.39	20

* A = One-half pint—left in refrigerator continuously; B = Quart—removed from refrigerator each day for sampling; C = Quart—allowed to stand out for 1 hour prior to storage, also removed from refrigerator each day for sampling; D = Quart—allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.

† Homogenized milk.

The retention of ascorbic acid during storage was very poor in most samples. Even the one-half pint samples which were refrigerated continuously and not exposed to light retained very little ascorbic acid after 1 day in storage. The exposure of samples to room temperature and light conditions for 2 hours caused an initial loss of about 20 per cent of this vitamin during the summer studies and about 12 per cent in the winter trials.

The high level of ascorbic acid found in the homogenized milk from one plant (samples 4 and 7, table 2) was difficult to explain. This milk was pasteurized at 170–172° F. for 16 seconds. Additional samples procured over a period of weeks showed that this heat treatment produced some sulphydryl compounds which protected ascorbic acid. Whereas most samples (homogenized or unhomogenized) were completely depleted of ascorbic acid within 4 to 6 days, most of these homogenized samples retained significant quantities of this vitamin throughout the entire storage period. The regular unhomogenized milk from this plant was pasteurized at a conventional temperature and ascorbic acid losses conformed with those experienced with milk from other plants.

Flavor Changes during Storage

During the winter months the samples in these studies exhibited somewhat better keeping qualities than did those in summer months. However, off-flavor development was somewhat more prevalent in the winter. Oxidized flavor was more frequently found during the winter trials than during the summer. Only one sample developed a typical oxidized flavor during the summer trials.

On the whole, the milks retained their initial fine flavor until very near the end of the storage period (0.03 per cent developed acid), when stale, unclean, acid or bitter flavor defects usually developed. With the exception of the occasionally observed oxidized flavor previously mentioned, practically all samples retained their initial flavors for at least 7 days. In several cases samples exhibited excellent flavor for more than 20 days of storage. Apparently the problem of flavor development during storage is relatively no more important when milk is consumed within a week after pasteurization than if consumed within 2 days.

DISCUSSION

One of the most interesting observations made in the course of these investigations is the apparent rôle of the psychrophilic bacteria in the deterioration of milk stored under refrigerator conditions. The initial mesophilic count (standard plate) appears to have little bearing on the potential keeping qualities of milks stored under the conditions of these experiments. Mesophilic counts at the termination of the storage period (0.03 per cent developed acid) frequently were little different from those exhibited by the fresh milk before storage. The suggestion that some mesophiles become adapted to storage conditions and eventually grow at these low temperatures seems justified, since mesophilic counts usually go down during the first few days of storage. This is further supported by the fact that rapid psychrophilic growth does not start until after 4 or 5 days of storage. The initial psychrophilic count does not appear to be a dependable index for predicting the potential keeping qualities of milk. The number of psychrophilic organisms in fresh pasteurized milk usually was found to be low (0–30 per ml.), but a considerably higher count never was found to prevent the product from exhibiting excellent keeping qualities.

The development of acidity during storage was found to correlate quite definitely with psychrophilic growth. The mesophilic populations present were comparatively small and probably contributed very little to the development of lactic acid under the conditions of these experiments.

The general growth curves for mesophilic, psychrophilic, and coliform organisms all followed like patterns. The magnitude of population change was much greater in the case of the psychrophiles and was by far the least for the coliforms. In the summer studies little change was observed in any of the bacterial counts until after 4 days of storage, while in winter important changes were not noted until after 7 days.

The flavor of the milks changed very little until near the end of the storage period (0.03 per cent developed acid), when stale, unclean, acid or bitter flavors usually developed. In several cases during the winter studies and in one case during the summer trials, the oxidized flavor developed soon after the milk went into storage. With these exceptions the flavor of the milks was exceptionally good until measureable lactic acid was produced. In very few cases was it possible to taste any evidence of acid until the concentration was in excess of 0.03 per cent.

The storage of milk in the dark at 40° F. did not change the riboflavin content of the product, and the periodic exposure of milk to the daylight in the laboratory had little or no effect upon this vitamin. Ascorbic acid was depleted very rapidly both before and after storage, only insignificant amounts of this vitamin usually remaining after 1 day of storage. One exception was found in the case of homogenized milk which had been flash pasteurized at 170–172° F. The protective effect of sulphydryl compounds produced by this pasteurization temperature accounted for the high levels of ascorbic acid observed in and retained by these samples in storage.

Good quality pasteurized market milks will maintain good bacteriological and flavor qualities for at least 4 days in summer and 6 to 7 days in winter, if refrigerator temperatures are maintained near 40° F. Exposure of milk to room temperatures naturally will impair the keeping qualities of the product, but with good distribution practices and prompt removal of the milk from the doorstep, milk should retain excellent quality for a considerable period of time.

SUMMARY

The psychrophilic bacteria which develop in milk during refrigerator storage are primarily responsible for the deterioration of the product. These organisms apparently are responsible for the development of the acid which is produced in milk during storage at 40° F.

The initial mesophilic (standard plate) and psychrophilic counts do not serve as an index of the potential keeping quality of milk being stored at about 40° F. Mesophilic counts frequently are little changed during the entire storage period.

Riboflavin in milk is not affected by refrigerator storage, and periodic exposure to room daylight has no noticeable effect.

The ascorbic acid content of milk is depleted rapidly both before and during storage. After 1 day of storage, only insignificant quantities remain in milks processed by conventional procedures.

Milk of good quality can be expected to retain excellent bacteriological and flavor qualities for at least 4 days during the summer months and 6 to 7 days during winter months if refrigerator temperatures are maintained near 40° F.

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COMPOSITION OF MARES' MILK AS COMPARED WITH THAT OF OTHER SPECIES[1]

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The skeleton of mammals calcifies very rapidly during the period immediately following birth when there is a rapid progressive penetration of calcium salts into the cartilaginous areas of the skeletal tissues. Furthermore, from an extensive review of the literature dealing with the requirement for calcium during growth of the human infant, Holmes (33) showed that the most rapid skeletal growth occurred during the initial period of the life cycle and that this probably is true for other mammals. Nearly 50 years ago Abderhalden (1) reported that the average foal doubles its birth weight in 60 days. During that period the foal subsists very largely on its mother's milk, and obviously all the mineral elements needed by the foal for bone and tissue building must be derived primarily from the mare's milk. However, very few data are available regarding the mineral content of mares' milk. A number of investigators have reported the amount of ash found in mares' milk: *i.e.*, Linton (39) found 0.28–0.95 per cent, with an average of 0.51 per cent; Vieth (67) found 0.26–0.36 per cent, with an average of 0.30 per cent in the milk from 15 milk mares; Hildebrant (24), 0.32–0.74 per cent; Papp (51), summer milk 0.3 per cent and winter milk 0.5 per cent; Dittrich (13), 0.29–0.60 per cent; and Masek (42), 0.35 per cent in the milk of the wild mare Przewalski kept in the zoological gardens in Prague. However, these data supply little or no information concerning the amount of various mineral constituents such as calcium, magnesium, phosphorus, and potassium in mares' milk, and the present study was undertaken to accumulate data regarding these elements.

EXPERIMENTAL

The mares' milk used in this study was produced by one Palomino and four Percheron mares. All the mares were mature, well-developed, normal animals. Their ages varied from 4 to 10 years and, numbered 1 to 5, consecutively, they were in their second, fifth, first, fifth and first lactations, respectively. The study was made in the spring. The mares' daily ration consisted of 3 quarts of crushed oats, five large ears of thoroughly matured dent corn, all the good-quality hay they desired, and as much rapidly-growing grass as they could eat in 3 or 4 hours. The Palomino mare, no. 1, weighed 1,100 lbs. and the Percherons from 1,600 to 1,900 lbs. The stage of lactation varied from the ninth day for the first sample of milk from

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mare no. 1 to the 129th day for the last sample from mare no. 5. While the stage of lactation was not the same for all the animals, the days on which the milk was collected were identical for all the mares. The samples of milk were collected as described by Holmes and Lindquist (28) and Holmes and associates (31). Samples were taken directly to the laboratory, thoroughly mixed, and assayed for water, protein, reduced ascorbic acid, phosphorus, potassium, magnesium, and calcium by the official methods of the Association of Official Agricultural Chemists (3). In order to minimize possible decomposition, the ascorbic acid assays were begun within an hour after the milk was obtained from the mares.

COMPARISON OF MARES' MILK WITH THAT OF OTHER SPECIES

The results of the assays of the 26 samples of mares' milk collected during the early lactation period are reported in table 1. Data concerning the composition of milk produced by several mammalian species are included in table 2. These data do not constitute all that are available in this field, but they should be sufficient to permit a comparison of the composition of mares' milk with that of other species.

The composition of the milk from each of the five mares varied somewhat from day to day. Since the values obtained for the Palomino mare's milk were not in close agreement with those obtained for the other mares, the average values for milk from the four Percheron mares have been used in the discussion that follows.

Water. The average values for the water content of the milk examined in this study ranged from 88.7 per cent for mare no. 2 to 90.2 per cent for mare no. 4. These values are in good agreement with the results obtained by the English investigators and are greater than that reported for cow, goat, ewe, buffalo, camel, or human milk. However, the difference in water content between mares' milk and that of other species is not sufficiently large to preclude a satisfactory comparison of the other constituents of the milks of the various species.

Protein. The amount of protein in the milk was quite uniform for the individual mares, but the average values for the different animals varied from 2.0 to 2.7 per cent. The average value of 2.3 per cent obtained in this study is higher than that reported by Vieth (67) and Morrison (44) but lower than the values reported by Linton (39) and Hildebrant (24). The protein content of the mares' milk was less than that of cow, goat, ewe, buffalo, camel, or sow milk, but it was similar to that of human milk, as reported by several investigators. In view of the similarity in protein and lactose content of mare and human milk, attention has been given to the possibility of using mares' milk for infant feeding. From his studies in this field, Frendenberg (20) found that because of its low fat content, mares'

TABLE 1

Composition of mares' milk

Mare no.	Day of lactation	Water	Protein*	Ascorbic acid	Phosphorus	Potassium	Magnesium	Calcium
		(%)	(%)	(*mg./l.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
1	9	88.2	2.9	62	78	74	10.8	126
	10	89.2	3.3	83	79	87	10.8	122
	11	89.2	3.3	88	85	88	11.8	131
	12	89.1	3.2	90	87	99	11.2	133
	13		3.3	90	90	91	14.7	107
	14	89.8	3.0	73	79	84	10.6	127
Av.		89.1	3.2	81	83	87	11.7	124
2	12	88.6	2.9	95	76	63	11.2	142
	13	88.4	2.7	94	61	46	9.1	111
	14	88.6	2.5	99	65	53	8.9	122
	15	88.9	2.6	101	71	58	9.7	135
	16	88.8	2.6	101	68	60	9.4	127
Av.		88.7	2.7	98	68	56	9.7	127
3	25	89.9	2.4	79	72	87	12.9	115
	26	89.6	2.5	83	74	86	12.9	112
	27	90.1	2.4	70	70	77	12.0	106
	28	89.9	2.3	73	69	76	9.7	102
	29	89.1	2.2	75	69	71	8.6	97
Av.		90.0	2.4	76	71	79	11.2	106
4	38	90.3	2.1	88	60	65	9.0	98
	39	90.0	2.1	88	59	66	9.3	97
	40	90.3	2.0	74	58	62	8.3	93
	41	90.2	2.1	70	58	56	8.9	94
	42	90.0	2.2	68	59	66	7.3	91
Av.		90.2	2.1	78	59	63	8.6	95
5	125	89.5	2.0	112	52	55	7.1	79
	126	90.1	2.0	103	50	50	6.4	80
	127	89.9	2.0	100	52	55	6.6	82
	128	90.1	1.9	108	52	62	6.2	78
	129	89.6	2.0	104	54	59	5.0	80
Av.		89.8	2.0	105	52	56	6.3	80
Av. Percherons (mares 2 to 5, inc.)		89.7	2.3	89	63	64	9.0	102

* Protein = N × 6.38.

TABLE 2

Comparison of mares' milk with that of other species

Source of milk	Water	Protein	Ascorbic acid	Ash	Phosphorus	Potassium	Magnesium	Calcium
	(%)	(%)	(*mg./l.*)	(%)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
Mare	89.04 (39) 90.13 (67) 90.70 (34)	1.65 (67) 2.06 (39) 2.55–3.13 (24) 3.00 (44)	27–115 (54) 87–197 (10) 95 (9)	0.51 (39) 0.30 (67) 0.32–0.74 (24) 0.30–0.50 (51) 0.29–0.60 (13) 0.35 (42)	50 (44)	80 (44)		80 (44)
Cow	86.21 (50) 87.90 (2) 87.10 (66)	3.20 (34) 3.13–3.77 (2) 3.50 (44) 3.30 (14) 3.20 (66)	21–22 (61) 18–20 (32) 25 (38) 20–25 (46) 18 (26) 16 (30) 12–15 (42) 16 (29)	0.69 (14) 0.70 (66) 0.72 (50) 0.68–0.74 (34) 0.70 (44)	68–92 (48, 49, 57, 58) 76–113 (11) 82 (37) 84–127 (63) 93 (61) 100 (36)	123–180 (12) 126–192 (52) 105–172 (2) 143 (60) 140 (44)	5–22 (48, 49, 57, 58) 10–12 (52) 11–17 (63) 7–12 (41) 10 (36)	90–155 (12) 94–150 (63) 96–161 (11) 102–148 (2) 111–132 (52) 118 (60) 120 (36) 155–171 (41)
Goat	87.14 (2) 85.71 (34)	4.29 (34) 3.40 (27) 3.70 (44) 2.99–3.71 (52) 3.99 (4)	5–20 (55) 20 (65) 17 (30) 13 (22) 85 (56) 9 (8) 45 (9)	0.31 (70) 0.94 (6) 0.78 (2) 0.81 (25) 0.81 (43) 0.78 (71) 0.79 (5) 0.80 (68)	98 (5) 124 (19) 96 (6) 104 (43) 112 (27) 100 (44)	193 (6) 171–228 (52) 106–242 (27) 150 (44)	14–20 (52) 13–22 (27)	114 (5) 131 (43) 138 (6) 141 (19) 137 (27) 130 (44) 117–150 (52)

TABLE 2 (*Continued*)

Source of milk	Water	Protein	Ascorbic acid	Ash	Phosphorus	Potassium	Magnesium	Calcium
	(%)	(%)	(*mg./l.*)	(%)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
Ewe	82.90 (2) 80.82 (34)	6.50 (44) 6.52 (34) 5.44 (2)		0.85 (2) 0.89 (34) 0.90 (44)	120 (44)	190 (44)		210 (44)
Buffalo	76.80 (2) 82.09 (2) 82.69 (34)	5.88 (34) 4.16–6.04 (2)	10 (8)	0.78–0.86 (2) 0.76 (34)				
Camel	87.61 (2)	2.98 (2)		0.70 (2)				
Sow		5.90 (44)		1.00 (44)				
Elephant				0.40 (64)				
Human	87.43 (23) 87.68 (6) 87.41 (34)	2.29 (34) 1.05 (7) 1.63 (23) 1.10 (17) 0.96 (18)	35 (8) 27 (9) 40 (16) 21–90 (53) 5–22 (69) 52 (45) 60–80 (59) 37 (35) 18–46 (62)	0.20 (19) 0.31 (70) 0.31 (6) 0.20 (7) 0.21 (43) 0.18–0.21 (71) 0.31 (34)	13 (43) 20 (19) 16 (47)			23 (19) 30 (47) 35 (43)

Figures in parentheses refer to the number of the reference cited.

milk could not be used successfully for infant feeding unless 1.0 to 1.5 per cent of cows' milk fat was added.

Reduced ascorbic acid. The average ascorbic acid content of the milk produced by the Percheron mares, 89 mg. per liter, was much higher than that previously reported by Holmes *et al.* (31), but was within the range reported by Cimmino (10) and by Rasmussen *et al.* (54), and was practically identical with the value reported by Cimmino (9). The reduced ascorbic acid content of fresh mares' milk was much greater than that of cow, goat, or buffalo milk. The reported ascorbic acid content of human milk varies over wide limits. Widenbaur and Kühner (69) found a minimum of 5 mg. per liter, and Quesada (53) reported as much as 90 mg. When Ingalls *et al.* (35) administered massive doses of ascorbic acid orally or intravenously to women, the milk subsequently secreted had an ascorbic acid content of 116 mg. per liter. A number of investigators (16, 35, 59) have increased the amount of ascorbic acid in human milk several-fold by adding ascorbic acid-rich foods to the diet. Furthermore, other workers found a marked seasonal variation. Sinkko (62) made observations on ten subjects in February and again in September and found 1.8 mg. and 4.6 mg. per liter, respectively. Other authors have found seven or eight times as much ascorbic acid in human milk in late summer as in the winter.

Ash. The reported assays for the amount of ash in mares' milk indicate that the average value is between 0.4 and 0.5 per cent. The mineral content of cow, goat, ewe, buffalo, and camel milk is 0.7 per cent. A number of investigators have reported upon the mineral content of human milk and the average value seems to be between 0.2 and 0.3 per cent. Thus mares' milk contains about two-thirds as much ash as the other species of animals cited and about twice as much as human milk.

Phosphorus. The phosphorus in the twenty samples of milk from the Percheron mares averaged 63 mg. per 100 g. This value is somewhat higher than the value reported by Morrison (44), but it is only about two-thirds as high as in cows' milk. Goats' milk has a slightly higher phosphorus content than cows' milk, but judged by the limited reports found in the literature, human milk contains only about one-fifth as much phosphorus as mares' milk.

Potassium. The average amount of potassium (64 mg. per 100 g.) found in the samples of Percheron mares' milk included in this study is somewhat less than the 80 mg. reported by Morrison (44). The potassium content of cows' milk, as indicated by the reports cited in table 2, is more than twice that of mares' milk. The amount of potassium in goats' and ewes' milk is appreciably higher than that of cows' milk and decidedly higher than that of mares' milk.

Magnesium. The magnesium content of mares' milk as obtained for the individual mares was fairly consistent, but the average values for the different animals varied from 6.3 mg. to 11.2 mg. per 100 g. The available literature supplied no data regarding the amount of magnesium in mares' milk. The mean result obtained for the four Percheron mares was 9 mg. of magnesium per 100 g. of milk. This definitely is less than the amount of magnesium present in cows' milk and goats' milk. No data were found concerning the amount of magnesium in human milk.

Calcium. The calcium content of milk, particularly that of cows and goats, has received considerable attention, since these milks are used most frequently as substitutes for human milk in infant feeding. The mares' milk was found to contain an average of 102 mg. of calcium per 100 g. This amount is significantly less than the amount present in cows' or goats' milk. However, it is three or four times the amount reported by Forbes and Keith (19), Nims *et al.* (47), and Maynard (43) for human milk.

Calcium-phosphorus ratio. Since calcium and phosphorus are required in much larger amounts than other minerals for bone formation, the ratio of calcium to phosphorus is of interest to those concerned with animal and human nutrition. The ratio of calcium to phosphorus in cows' milk has been reported as 1.40 (37), 1.20 (36), and 1.27 (60); in goats' milk as 1.44 (6), 1.26 (43), 1.22 (27), 1.14 (19), and 1.16 (5). The values noted in the literature for the ratio of calcium to phosphorus in human milk were very limited and extremely variable: *i.e.*, 2.69 (43), 1.84 (47), and 1.25 (19). The ratio of calcium to phosphorus obtained for mares' milk was 1.62, a value which is definitely higher than that of cows' or goats' milk but possibly lower than the calcium-phosphorus ratio of human milk. It may be noted that Linton (40) has reported that the calcium and phosphorus content of mares' milk decreased linearly with the advance of lactation, but the calcium-phosphorus ratio remained quite constant throughout the lactation period.

SUMMARY

Twenty-six samples of milk produced by one Palomino and four Percheron mares were assayed for water, protein, ascorbic acid, phosphorus, potassium, magnesium, and calcium. The milk was produced in the early lactation period during late winter and early spring months when the mares were subsisting principally upon hay and grain. The milk from the Palomino mare contained more protein, phosphorus, potassium, and magnesium than the Percheron mares' milk. The average values for the milk of the Percheron mares were: water 89.7 per cent, protein 2.3 per cent, reduced ascorbic acid 89 mg. per liter, phosphorus 63 mg., potassium 64 mg., magnesium 9.0 mg., and calcium 102 mg. per 100 g. These values indicate that mares' milk contains more water than cow, goat, ewe, buffalo, camel, or human milk; less protein than cow, goat, ewe, buffalo, or camel milk, but more than human milk; more ascorbic acid than cow, goat, or human milk;

less phosphorus than cow or goat milk but more than human milk; only about one-third as much potassium as cow or goat milk; and less magnesium and calcium than cow or goat milk, but about four times as much calcium as human milk. The ratio of calcium and phosphorus is considerably higher in mares' milk than in cows' or goats' milk but possibly lower than in human milk.

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FLAVONES AND FLAVONE DERIVATIVES AS ANTIOXIDANTS

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The demands of war lent encouragement to the search for acceptable inhibitors of oxidative deterioration of food fats. This impetus resulted in the discovery of many effective processes and chemical agents, most of which have been patented but few of which have been acted upon by the Food and Drug officials.

The literature dealing with antioxidants has been covered by several reviews (6, 8, 9, 22, 26, 36, 37) and the characteristics and limitations of antioxidants and synergists have been discussed by Mattill (32), Golumbic (14), and others.[2] A partial list of antioxidants has been prepared (2).

The resistance of dry milk fat to oxidation has been shown to be due in part to its content of reducing substances (11) as determined by the Emmerie-Engel reagent. This reagent was found to be non-specific for α-tocopherol, a fact now universally recognized. It recently has been modified to determine the solubility in fats of several phenolic antioxidants (29). Experiments with this reagent suggested that reducing substances or components of oxidation-reduction systems that occur naturally in foods and that have food value in themselves should be ideal for incorporation into fats or fat-containing foods to serve as protectors against oxidation.

Reducing substances have been found among citrus juices, citrus peel, flower petals, rose hips and many other naturally occurring materials. Shrader and Johnson (44) recognized in orange juice distinct zones of oxidizing and reducing effects, the reducing effects being associated with the pigments. Svirbely and Szent-Györgyi (45) attributed part of the reducing value of orange juice to phenolic compounds later identified as flavonols. Hamburger and Joslyn (18) and Joslyn and Marsh (23), in studies related to browning of citrus juices, considered that among the reducing substances present in the juice were ascorbic acid and flavonols which might serve as a defense against browning. They believed that all of the reduced ascorbic acid first must be oxidized before the unknown reducing substance can itself be oxidized. This is in harmony with Golumbic's view (14). The flavones are known to oxidize ascorbic to dehydroascorbic acid (24).

Frankenthal (12), investigating the methylene blue reducing system of Palestine orange peels, found that the peel juice contained two factors not

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[2] B. F. Daubert and H. E. Longenecker (Food Technology, 1(1): 7–10, 1947) recently have given a comprehensive discussion of the rôle of antioxidants in flavor problems.

adsorbed on animal charcoal and also an iodine-reducing, heat-stable factor which is adsorbed on animal charcoal and which is not ascorbic acid. The author recognized an oxidizing system in peel juice which acted upon ascorbic acid. Supporting evidence that flavone or flavone-like pigments contribute to the total reducing power of orange and raisin juices was contributed by Gatet (13), who studied the reducing power of quercetin, quercitrin, and their oxidation products. The impurity accompanying the flavones was thought to be a contributing factor. The reducing activity of these juices and of the flavone-type solutions, as measured by the 2-6,dichlorophenolindophenol method, was increased by first oxidizing in air at pH 8, followed by reduction by cysteine at pH 4. This may help to explain the beneficial results obtained by Shrader and Johnson (44) when oxygen was bubbled through certain batches of orange juice prior to packaging.

The belief that the flavone and flavanone pigments participate in oxidation-reduction reactions is strengthened by the work of Wawra and Webb (48) with citrin in which evidence was presented to show that citrin consists of hesperidin and the chalcone of hesperidin existing in equilibrium in an aqueous medium. In nature they probably exist as protein complexes and serve as an oxidation-reduction enzyme. The chalcone was shown to be capable of being reversibly oxidized and reduced. A small amount of quercitrin is believed to accompany the citrin, especially in the citrus fruit (41).

A review of the literature indicates that no attempt has been made to utilize the flavones or other phenyl-benzopyrone derivatives as antioxidants for animal fats. Greenbank and Holm (16) reported that quercetin seemed to possess antioxidant properties for cottonseed oil; Bradway and Mattill (5) found quercetin from quercitrin to be antioxygenic for a mixture of lard and cod-liver oil.

From the physiological, pharmacological, and nutritional aspects, the addition of flavones and their derivatives to foods appears acceptable and beneficial. They have been reported as vitamin P in a wide variety of foods (3, 38, 43, 47) following the postulation of Szent-Györgyi (46) that citrin, a mixture of flavones, possessed vitamin P activity. Many workers have used the biological technique to determine the vitamin P potency of fruits and vegetables (3, 41, 42); others have employed the chemical method of Lorenz and Arnold (28). Weatherby and Cheng (49) utilized the boric-citric acid reagent and reported the flavone or quercetin-like values of food products in terms of quercetin equivalents. Lemon peel ranked highest among the materials tested.

The occurrence, chemical nature, and vitamin P activity of several members of the flavone group have been reviewed (20, 27, 43). Direct evidence that the flavonol quercetin enhances the nutritive value of butter oil has been reported by El-Rafey *et al.* (10). This is in harmony with the growing appreciation of the role of antioxidants in fat, vitamin A, and carotene utilization.

Regardless of whether the flavone-type compounds complement the physiological activity of ascorbic acid, there seems to be accepted evidence that most of the representative compounds studied have beneficial pharmacological effects. The authors feel incompetent to analyze the extensive medical literature dealing with use of these compounds as therapeutic agents. They appear to have little or no toxicity, to be non-accumulative (17), and to have nutritional and medical significance (1, 31).

EXPERIMETAL

Substrates. Dry milk fat or butter oil was prepared by churning fresh pasteurized cream, melting the butter at 60° C., and decanting and filtering the resulting fat at 60° C. This fat was divided into small portions and refrigerated until required. The lard was prepared by melting at 60° C. fresh pure lard, obtained in the local market, filtering and refrigerating. For the experiments with milk, 2 p.p.m. of copper as copper sulfate were added to pasteurized winter milk, susceptible to copper-induced oxidation.

Antioxidants. These contained the phenyl-benzo-gamma-pyrone nucleus, usually with hydroxyl, glyco, or methoxy groups or the chalcone isomer. Quercetin, quercitrin, rutin, hesperidin, methylated hesperidin, hesperidin-chalcone, and others of the flavone group were studied.[3] Attempts to isolate and purify the individual components of Szent-Györgyi's citrin (46) were unsuccessful. Difficulty was experienced in separating and eluting the fractions in the Mager method (30). Recent methods should prove more fruitful (21, 39).

Incorporation of antioxidants. Most of the antioxidants studied were more soluble in alcohol than in water or fat and hence were dissolved in either hot ethanol or glycerol before being incorporated into either a small quantity of fat in a relatively high concentration or directly into the fat in the concentration desired. The alcohol then was evaporated off in partial vacuum at from 90 to 96° C. In the experiments with milk, 20 mg. of the antioxidants dissolved or suspended in 5 ml. of hot water were added to 200 ml. of milk. The excess of the material settled out during the storage period.

For the experiments in which quercetin was compared with antioxidants native to milk fat, stock solutions were prepared as follows:

A. *Soya bean phospholipid.* Commercial soya bean lecithin[4] was purified by dissolving twice in ether and precipitating with acetone. Eight grams, dissolved in 5 ml. of chloroform, were added to 400 ml. of milk fat to give a 2 per cent solution.

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[3] Quercetin, Eastman Kodak Co. 1635; quercitrin, Eastman Kodak Co. T1629, purified (34); rutin, courtesy of Dr. J. F. Crouch, Eastern Regional Res. Lab., Philadelphia; hesperidin-chalcone, methylated chalcone, and hesperidin, courtesy of California Fruit Growers Exchange.

[4] Courtesy of Dr. Eichberg, American Lecithin Company, Elmhurst, New York.

B. *Tocopherol.* 0.1 g. α-tocopherol, dissolved in 1 ml. of chloroform was added to 100 ml. of milk fat to give a 0.1 per cent solution.

C. *Quercetin.* 0.1 g. quercetin, dissolved in 2 ml. hot absolute alcohol was added to 100 ml. of milk fat to give a 0.1 per cent solution.

The solvent was removed as described.

Incubation. Fat stabilities were determined by the open jar method, in which a uniform amount of the fat is placed in similar containers and incubated uncovered. The temperature conditions were secured by a controlled oil bath, electric oven, or refrigerated cabinet. The cabinet was maintained at approximately 10° C. for the milk samples.

Tests for stability. Peroxide values were determined by the Henderson and Young modification (19) of the Wheeler method (50). Carotene losses were measured as decreases in optical density (10% solution of the fat in gasoline, 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.) using the Coleman, Model 11, spectrophotometer. The milk samples were scored for flavor as unknowns.

RESULTS

Effect of concentration of antioxidant. Preliminary trials having shown that quercetin is antioxygenic for milk fat and for lard, experiments were made to determine an effective concentration for its use. Figures 1 and 2 show that a concentration of 3 mg. per 100 g., while affording protection, is not as adequate as a concentration of 15 or 30 mg. per 100 g. at an incubation temperature of 82° C. The figures also show that the incorporation of 15 mg. quercetin in 100 g. fat extended the times required to reach a peroxide value of 5 for milk fat or 10 for lard (respective arbitrarily chosen ends of the induction periods) from 30 to over 144 hours for milk fat and from 26 hours to over 96 hours for lard. Competent judges were unable to detect by taste or color the presence of at least 30 mg. per cent of quercetin in butter oil, lard, or butter-like preparations made from them.

Protection of milk fat in storage. It was shown previously (11) that soya bean phospholipids and α-tocopherol, either alone or in synergistical combination were antioxygenic for butter oil held at 79.5° C. In order to determine the effectiveness of these compounds at a lower temperature and to compare their effectiveness with that of quercetin, a milk fat was isolated in the manner described except that melting and filtration were carried out at 41–47° C. rather than at 60° C.

Using the stock solutions A, B, or C, or combinations of A and B, samples of this fat were prepared to contain from 0.01 to 0.1 per cent added phospholipid (mostly lecithin), from 0.003 to 0.015 per cent added α-tocopherol, or from 0.003 to 0.015 per cent quercetin. Synergistic combinations were prepared containing 0.01 per cent phospholipid and 0.003 per cent α-tocopherol; 0.1 per cent phospholipids and 0.003 per cent α-tocopherol; or 0.1 per cent phospholipid and 0.015 per cent α-tocopherol, in addition to those naturally present.

With the exception of samples 1 and 4 which were stored in test tubes

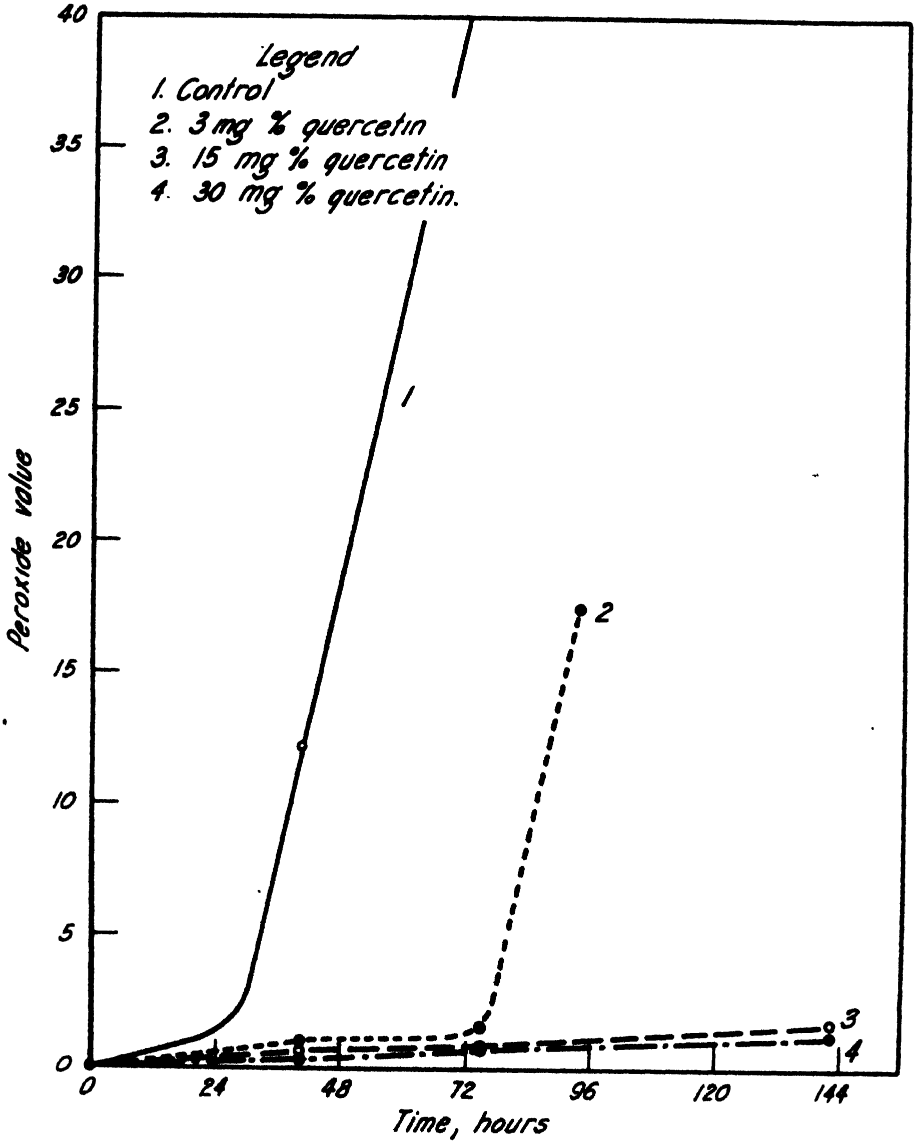

Fig. 1. The effect of adding quercetin to butter oil on its resistance to oxidation at 82° C.

at 4.5–10° C. (40–50° F.), approximately 100 ml. aliquots of each sample, contained in one-quarter pint milk bottles, with loosely-fitting caps, were stored at 40–50° F. from July 27, 1943, until about October 1, 1943. They then were placed in a wooden cabinet at room temperature. At intervals the fats were melted and samples were taken for peroxide and color determinations. The results are shown in table 1. The experiment does not lend itself to an evaluation of absolute protection factors of the antioxidants, but the data suggest that α-tocopherol added to milk fat, normally containing tocopherol (2–3 mg./100 g.), has no protective value under these conditions

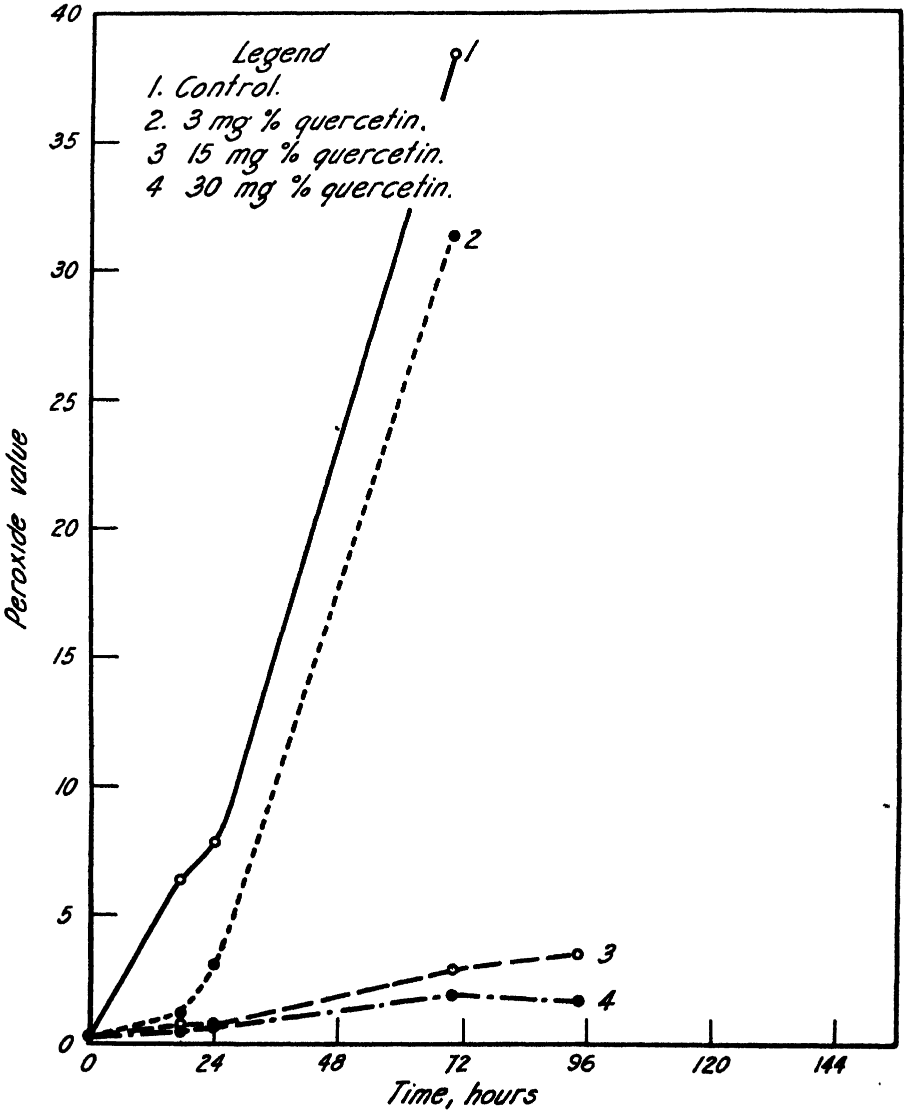

FIG. 2. The effect of adding quercetin to lard on its resistance to oxidation at 82° C.

TABLE 1

Effect of antioxidants on the storage life of milk fat

(Samples were prepared in July, 1943, held cold until Oct., 1943, and subsequently stored at room temperatures)

Sample no.	Description	Peroxide no.* and optical densities† (in parentheses)					
		Feb. 5, 1944	June 2, 1944	Sept. 26, 1944	May 7, 1945	Sept. 8, 1945	July 22, 1946
1	Control, milk fat held at 40–50° F.		0.75 (0.255)	2.5	70 (white)		
2	Milk fat plus 0.01% P‡	0.96	1.91 (0.243)	3.45 (0.21)	(white) (0.075)		
3	Milk fat plus 0.05% P	0.63	0.73 (0.26)	2.35 (0.24)	18.4 (0.05)		
4	Milk fat plus 0.1% P held at 40–50° F.		0.0 (0.26)	0.0 (0.26)	1.52		
5	Milk fat plus 3 mg.% T‡	1.1	3.71 (0.20)	8.16 (0.15)	(white)		
6	Milk fat plus 9 mg.% T	1.7	6.39 (0.23)	12.15 (0.13)	(white-0.08)		
7	Milk fat plus 15 mg.% T	2.94	14.5 (0.255)	16.26 (0.115)	(white)		
8	Milk fat plus 0.01% P and 3 mg.% T	0.37	1.3 (0.27)	3.56 (0.22)	(white-0.075)		
9	Milk fat plus 0.1% P and 3 mg.% T	0.24	0.92 (0.264)	2.83 (0.23)	Almost white		
10	Milk fat plus 0.1% P and 15 mg.% T	0.48	1.98 (0.256)	7.1 (0.23)	(0.07)		
11	Milk fat plus 3 mg.% Q‡	0.55	0.83 (0.275)	1.33 (0.25)	3.88 (0.205)	6.43	
12	Milk fat plus 15 mg.% Q	0.24	0.53 (0.31)	0.89 (0.29)	1.96 (0.30)	2.46	3.18
A	Milk fat plus 2% P			0.0 (0.27)	0.84 (0.25)	2.02	3.83
B	Milk fat plus 0.1% T			35.1 (0.21)	(0.06)		
C	Milk fat plus 0.1% Q			0.0 (0.29)	0.82 (0.265)	1.44	1.35

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* Millequivalents per kg. of fat.

† Optical density (10% solution of fat in gasoline: 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.)

‡ P—phospholipid; T—α-tocopherol; Q—quercetin.

of storage. It even appears prooxygenic. Soya bean phospholipid (mainly lecithin), of itself and in combination with α-tocopheról, is definitely antioxygenic for milk fat. The proportion of 0.003 per cent tocopherol to 0.1 per cent phospholipid (sample 9) appears a desirable addition.

The phospholipid content of the control fat was 0.0214 per cent (calculated as lecithin). Assuming that milk fat contains 0.0025 per cent tocopherol, sample 9 had a tocopherol: phospholipid ratio of approximately 1:22, whereas the ratio for the control fat was approximately 1:8.6.

The effectiveness of quercetin, especially in the 0.015 per cent concentration, is outstanding. The 0.1 per cent concentration, while effective, is in considerable excess of the solubility of quercetin in fat. The protective action of quercetin for carotene will be the subject of a later paper.

The relative effectiveness of quercetin and commercial quercitrin. Quercetin and quercitrin (unpurified), Eastman Kodak Co., were added to milk fat and to filtered lard colored with carotene by the addition of 0.4 ml. of a concentrate[5] to 200 ml. lard. After removal of the solvent, ethanol, 50 ml. samples, in 200-ml. straight-walled bottles, were incubated in the dark at 47–50° C. Table 2 shows that quercetin is more effective than quercitrin on the weight basis. The latter is a quercetin 3-rhamnoside and has a quercetin equivalent of 354 mg. per gram as determined by the method of Weatherby and Cheng (49). On this basis, then, the quercitrin was present only in a quercetin-equivalent concentration of approximately 7 and 10.5 mg. per cent in the milk fat and lard, respectively.

Other flavone-type compounds as antioxidants. Following the precedure suggested by Wawra and Webb (48), crude preparations of hesperidin and its chalcone were made from air-dried orange and lemon peels. Their identity and purity were not established. The compounds, dissolved in ethanol, were incorporated by the described procedure, but the samples were subjected to a fluctuating temperature of 72–75° C. for the first 72 hours, except for about 6 hours at 90° C. They then were incubated at 48–50° C. The results in table 3 show that rutin from tobacco (quercetin-equivalent approximately 300 mg. per gram) has lower antioxygenic properties than quercitrin. Sample 4, containing rutin and a crude mixture of hesperidin and its chalcone, had a pronounced fruity odor after the first heating. This largely disappeared as incubation progressed. The sample showed remarkable resistance to the accumulation of peroxides. Sample 6 indicates the potential antioxygenic properties in the chalcone. This is demonstrated more conclusively in table 4. Partially purified flavanones (hesperidin, hesperidin-chalcone, methylated hesperidin-chalcone) and a lemon peel infusion concentrate prepared by the method of Szent-Györgyi for vitamin P studies were kindly furnished by California Fruit Growers Exchange. These were incorporated into milk fat in the manner already described, and

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[5] Courtesy of H. M. Barnett, Barnett Labs., Long Beach, California.

TABLE 2

Effect of quercetin and quercitrin on the keeping quality at 47–50° C. of milk fat and of lard containing carotene

(Approximately 9,000 I.U. vitamin A per lb.)

Sample no.	Description	Peroxide values and optical densities* (in parentheses)									
		Dec. 17, 1943	Dec. 23, 1943	Dec. 27, 1943	Dec. 31, 1943	Jan. 11, 1944	Feb. 2, 1944	Mar. 2, 1944	Mar. 25, 1944	Mar. 28, 1944	Apr. 7, 1944
B	Milk fat untreated	(0.30)	0.26								
L	Lard plus carotene untreated	(0.41)	0.65								
1‡	B-control	(0.28)	0.48 (0.28)	3.7 (0.22)	22.8 (0.06)	110.7 (0.02)	217 (0.02)				
3	B plus 20 mg.% QT†	(0.30)	0.86 (0.32)	1.94 (0.34)	1.91 (0.29)	2.61 (0.27)	35.4 (0)				
4	B plus 20 mg.% Q	(0.31)	0.36 (0.33)	(0.35)	1.03 (0.30)	1.9 (0.33)	3.44 (0.16)	3.72 (0.11)	6.47 (0.1)	25.4 (0.05)	113 (0.01)
5	L-control	(0.39)	1.04 (0.38)	14.9 (0.09)	43.3 (0.03)	97.4 (0.09)	150 (0.02)				
7	L plus 30 mg.% Q	(0.50)	0.88 (0.52)	(0.40)	1.41 (0.47)	2.61	3.54 (0.31)	4.98 (0.21)	7.68 (0.19)	9.49 (0.11)	15.3 (0.08)
8	L plus 30 mg.% QT	(0.50)	2.02 (0.51)	2.07 (0.50)	3.67 (0.43)	25.36 (0.16)	136 (0.04)				

* Optical density (10 per cent solution of fat in gasoline; 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.).

† Q—Quercetin; QT—quercitrin (not purified).

‡ Samples 1 and 5 received the same pre-incubation treatment as samples 3, 4, 7, and 8.

TABLE 3

Effect of quercitrin, rutin, and hesperidin chalcone on the keeping quality of milk fat at 40–50° C. (1944)

Sample no.	Description*	Peroxide values (me/kg. fat)										
		May 12	May 15	May 17	May 23	May 26	June 9	June 15	June 23	June 30	July 24	Oct. 4
1	QT 30 mg.%	1.37	1.58	1.60	1.93	2.11	2.66	3.0	3.41	3.49	6.72	
2	R 30 mg.%	1.71	2.35	2.46	3.4	12.94						
4	R 30 mg.% plus HC	0.0	0.0	0.0	0.0	0.0						
5	R 20 mg.% (dry)	1.87	2.26	2.64	15.4	42.2					0.8	3.36
6	Control	2.03	9.39	23.5								
7	HC conc. not determined	1.33	1.93	1.93	2.29	2.52	18.5					

* QT—quercitrin from lemon flavine (34); R—rutin from tobacco; HC—hesperidin chalcone, a purified extract of orange and lemon peels.

TABLE 4

Hesperidin and hesperidin chalcone as antioxidants for milk fat

(1945)

		Peroxide values (me/kg. fat)									
	Initial Oct. 2 3 p.m.	Oct. 3		Oct. 4		Oct. 5		Oct. 6 11 a.m.	Oct. 10	Oct. 12	Oct. 16
		1:20 p.m.	4:15 p.m.	9 a.m.	3:45 p.m.	11 a.m.	4 p.m.				
Experiment 1											
Control	1.7	2.32	2.41	7.04							
HC, 10 mg./30 g. fat				4.81	9.8	11.95					
HC, 20 mg./30 g. fat				2.62	2.48	3.98	6.24	13.75			
Quercetin 10 mg./30 g. fat				2.08	1.96	2.11	1.91	2.82	2.87	3.52	4.88
	Initial Oct. 4 3 p.m.	Oct. 5		Oct. 6 11 a.m.	Oct. 8 4 p.m.	Oct. 9 4 p.m.	Oct. 10 4 p.m.				
		10 a.m.	3:45 p.m.								
Experiment 2											
Control	1.31	2.5	3.96	6.9	8.32	13.25					
Crude H ca. 50 mg./30 g. fat		2.45	2.29	4.54	7.58	9.54	13.15				
Lemon peel infusion conc. ca. 40 mg./30 g. fat		2.23	2.41	2.73	2.76	6.35	10.11				

HC—Hesperidin chalcone—Courtesy California Fruit Growers Exchange.
H—Hesperidin, crude—Courtesy California Fruit Growers Exchange.
Lemon peel infusion for vitamin P studies—Courtesy California Fruit Growers Exchange.

the fat samples were incubated at from 60 to 80° C. during the day and chilled during the night or over the weekend. The results indicate that hesperidin has little or no antioxidant value. Other experiments, not reported, showed that the methylated chalcone probably is too stable to serve as an antioxidant. The data, however, show that the natural chalcone protects milk fat against oxidation.

Inhibiting oxidation in susceptible milk. Twenty mg. of the compounds listed in table 5 were added to 200 ml. of warm pasteurized susceptible milk along with 2 p.p.m. of copper. A control was prepared omitting the compound; a key sample, containing no added copper, was included. All were cooled and placed in a refrigerated cabinet (10° C.) and scored as unknowns on the third day. The scores and comments of one of the judges, concurred in by the other judges, are shown in the table. All of the flavones and flavanones studied inhibited the development of the oxidized flavor common to winter milk. Experiments are contemplated in which the antioxidant will be incorporated prior to pasteurization.

TABLE 5

Flavone-type compounds as antioxidants for milk susceptible to copper-induced oxidation

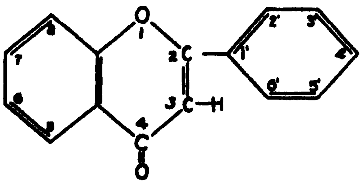
Sample no.	Antioxidant	Numerical score*	Comments
1	Key, no copper	21	Old, very sl. oxidized
2	Hesperidin	18	Oxidized
3	Hesperidin-chalcone-protein complex†	21	Sl. foreign
4	Rutin	21	Old
5	Quercitrin	22	Lacks freshness
6	Control, with copper	18	Oxidized
7	Lemon peel infusion concentrate (vitamin P)	20	Old, sl. oxidized
8	Quercetin	22	Old, lacks freshness
9	Same as 7, except one-half concentration	19	Oxidized
11	Hesperidin chalcone	20	Foreign flavor

* Flavor and odor; 25, no criticism.

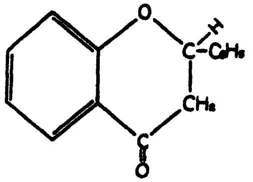
† Prepared by method of Wawra and Webb (48).

DISCUSSION

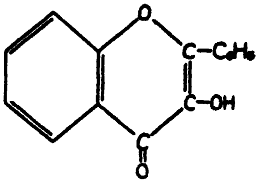
The flavones and flavone derivatives are distributed widely in the plant kingdom and occur as glycosides, in the free state, and in association with proteins and tannins. They all have the basic phenyl-benzo-gamma-pyrone structure, the pyrone nucleus apparently being responsible for their characteristic chemical activity. The key structures, according to Mayer and Cook (33), are as follows:



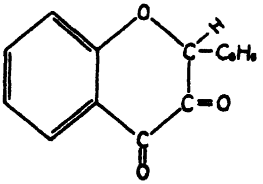
Flavone



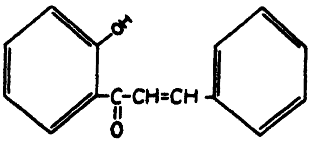
Flavanone



Flavonol
(enol form)



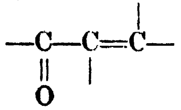
Flavonol
(keto form)



Chalcone

Mayer and Cook state that a flavanone may be dehydrogenated to a flavone and a chalcone may be converted to the flavonol by treatment with hydrogen peroxide. The chalcone in acid medium is believed to isomerize into the flavanone, the reverse taking place in the alkaline medium (48).

Of the flavone-type compounds studied, those giving a positive borocitric test (51) were most efficient as antioxidants. This test apparently involves the



$-\underset{\underset{O}{\|}}{C}-\underset{|}{C}=\overset{|}{C}-$

group. Quercetin (3,5,7,3',4', pentahydroxyflavone), quercitrin (3-rhamnoside of quercetin), rutin (3-rutinoside), and possibly the chalcone of hesperidin (48) all contain this grouping and all have antioxidant activity. It would seem logical, therefore, to infer that the labile pyrone confers antioxidant properties. The closed, saturated ring of hesperidin apparently does not satisfy this condition and it has very little, if any, antioxidant value, at least in the absence of a synergist of lower oxidation potential.

A theoretical discussion of the manner in which flavones and their derivatives protect a fat against oxidation is somewhat premature. Milk fat as usually isolated is afforded some protection by the phosphatides, tocopherols, and other reducing substances which it contains. These are difficult to remove without effecting changes in the fat itself. Carotene cannot be ignored. In milk itself the natural oxidation-reduction system or systems is not a simple one, even if ascorbic acid is excluded. Furthermore, individual flavones and flavanones are not easily isolated from their isomers and other naturally-occurring pigments.

The data are interpreted as supporting the modern concept of antioxidants. Very little seems to be known of the oxidation-reduction potentials of the flavones in a fat medium. The potential of a fat peroxide is not known, but that of a fresh fat may be about 1.0 volt (15). Gatet (13), by oxidizing quercetin in air at pH 8, followed by reduction with cysteine at pH 4, increased its apparent concentration toward 2,6-dichlorophenolindophenol about four-fold. It would seem that the potential of this activated quercetin in a water-alcohol medium at pH 7 is somewhat less than 0.22 volt. If this is true, sulfhydryl compounds and possibly ascorbic acid might well be expected to act synergistically with these flavones. Sample 4, table 3, suggests that a combination of a flavone and a chalcone may be very effective in inhibiting the accumulation of peroxides. This aspect is being studied.

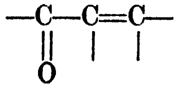
It is not known if ingested flavones or flavanones will be deposited in the body fat, carried in the blood stream, or secreted in the milk of animals, as appears to occur under certain conditions with tocopherol (7). None was found in the livers of rabbits (51) or in milk (35), and Robeznicks (40) was unable to establish either the presence or absence of flavones in the liver, kidney, or milk of animals. Feeding citrus molasses or dried citrus pulp to dairy cows has proved satisfactory and no ill effects on the milk flavor have been found (4, 25). If ingested flavones are found to be deposited in the body fat and secreted in milk, the authors are of the opinion that these ingested compounds would result in enhanced stability of meat products and improved keeping quality of winter milk and of whole milk products.

The lack of specific tests for flavones and flavanones, especially in the presence of interfering substances, coupled with the difficulty of securing foodstuffs free from flavones, makes conclusive results hard to obtain.

CONCLUSIONS

1. Flavones, as illustrated by quercetin, quercitrin, and rutin, have been shown to be effective antioxidants for milk fat and lard.

2. The flavanone glycoside hesperidin appears to have little or no antioxidant values; its chalcone is active.

3. It is suggested that the  $-\underset{\substack{\| \\ \mathrm{O}}}{\mathrm{C}}-\underset{|}{\mathrm{C}}=\underset{|}{\mathrm{C}}-$ group in the pyrone ring or in the open chalcone is responsible for the antioxidant activity.

The helpful suggestions of Professors G. Mackinney and H. G. Reiber in reviewing the manuscript, and the assistance of Professor C. L. Roadhouse and Mr. C. A. Phillips in the organoleptic tests are acknowledged.

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VARIATION IN THE CONSTITUENTS OF MILK UNDER ARIZONA CONDITIONS. I. VARIATIONS OF INDIVIDUAL COWS WITHIN BREEDS BY CALENDAR MONTHS

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Various investigators have found that the composition of milk varies throughout the year. These variations have become increasingly important to the producer. There have been instances in which milk produced in Arizona was represented as being below the legal requirement in serum solids but well above the minimum in fat. Both fat and serum solids often drop below the prescribed legal minimum, as shown by Overman, Sanmann, and Wright (7), with minimum serum solids values for Jersey, Guernsey, and Holstein milk of 7.68, 8.10, and 7.82 per cent, respectively.

Various investigators (2, 5, 8, 12, 14, 15) have studied seasonal variations in the composition of milk and have found that the butterfat percentage is lowest in summer and highest in winter. They have concluded that the butterfat test varies inversely with environmental temperature. Clothier (2) reported characteristic changes in fat content of Arizona milk without the corresponding decrease in environmental temperature. Hays (4) concluded from a 258-day operation that low environmental temperature is a major factor in the increase in percentage of fat. Regan and Richardson (9) also noted decreases in milk production, percentage serum solids, and casein content of the milk when the environmental temperature was raised.

The data of Overman *et al.* (7) show that of 199 samples of milk from fifteen Jersey cows, five samples (2.5 per cent), all from one cow, contained 7.68 to 8.23 per cent serum solids and 3.28 to 4.03 per cent fat, respectively; of 321 samples from sixteen Guernsey cows, none had tests below the 3.25 per cent minimum for butterfat and four samples (1.2 per cent), from two cows, had 8.19, 8.28, 8.45, and 8.34 per cent serum solids and 3.88, 3.90, 4.40, and 4.56 per cent fat, respectively; of 268 samples from nineteen Holstein

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cows, 85 (31.7 per cent) from twelve cows were below the minimum for butterfat and 24 samples (8.9 per cent) with 3.25 per cent or higher fat had less than 8.50 per cent serum solids.

EXPERIMENTAL

This study is concerned with the variations in the constituents of milk from individual, purebred Jersey, Guernsey, and Holstein cows and offers hitherto unpublished data on constituents of milk from Arizona cows. The milk used in this study was from cows in the herd of the University of Arizona. Samples were taken of the milk of each cow 1 day each month during the lactations over a period of 30 months. Representative samples were taken of the night and morning milk and then brought directly to the laboratory.

Determinations were made of percentage of butterfat, total solids, lactose, and protein. The percentage of butterfat was determined by the Babcock method; the total solids by the Mojonnier method; and the serum solids were calculated by difference. Total nitrogen was determined by the Kjeldahl method; the total nitrogen percentage then was multiplied by the factor 6.40 to obtain the percentage of protein. The polariscope was used to analyze for lactose. The serum solids of samples of milk shown in table 2, column 2, were calculated by the method of Jacobson (6). The method is based on data of over 100,000 herd samples of milk. The serum solids in the skim milk obtained from the sample and from normal milk were calculated by dividing the serum solids of the milk by 100 minus the fat percentage of the milk as shown by Sommer (12).

Figures 1 to 3, inclusive, give the average monthly values of each constituent for the three breeds studied. The data collected over a period of 30 months represent analyses of milk from eleven Jersey, eleven Guernsey, and twelve Holstein cows. At the beginning of the study the number of cows in their first to sixth lactations, respectively, were: Jersey, 5, 2, 2, 1, 1, 0; Guernsey, 7, 2, 0, 1, 1, 0; and Holstein, 4, 2, 1, 4, 0, 1.

TABLE 1

Maximum, minimum, and average values for milk constituents of individual cows of three breeds

Breed No. of samples	Jersey 160			Guernsey 139			Holstein 207		
	Max.	*Min.*	*Av.*	*Max.*	*Min.*	*Av.*	*Max.*	*Min.*	*Av.*
Lbs. milk daily	43.5	7.3	23.5	54.8	11.5	26.9	82.3	11.9	40.0
% Total solids	17.2	11.8	14.6	16.6	12.1	14.2	14.7	10.5	12.1
% Serum solids	11.3	7.9	9.5	11.5	7.4	9.4	10.3	7.4	8.6
% Fat	7.8	3.4	5.1	6.7	3.1	4.9	7.8	2.6	3.5
% Lactose	5.7	4.0	4.7	5.5	4.2	4.9	5.5	3.7	4.8
% Protein	4.1	3.2	3.7	4.5	2.9	3.7	4.0	2.6	3.0
Ratio of serum solids to fat	2.96	1.20	1.85	2.96	1.36	1.92	3.08	1.20	2.44

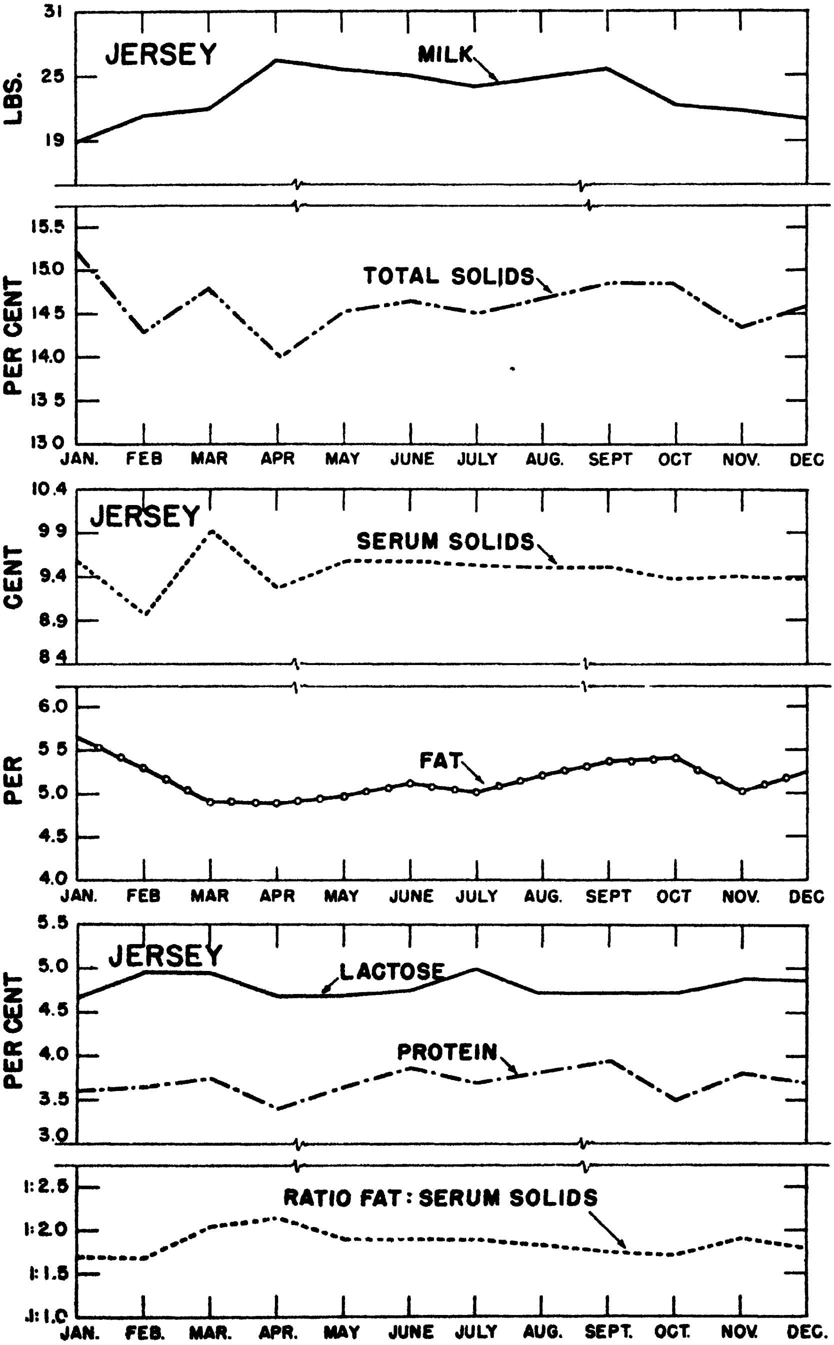

FIG. 1. Variations in constituents of milk from Jersey cows by calendar months.

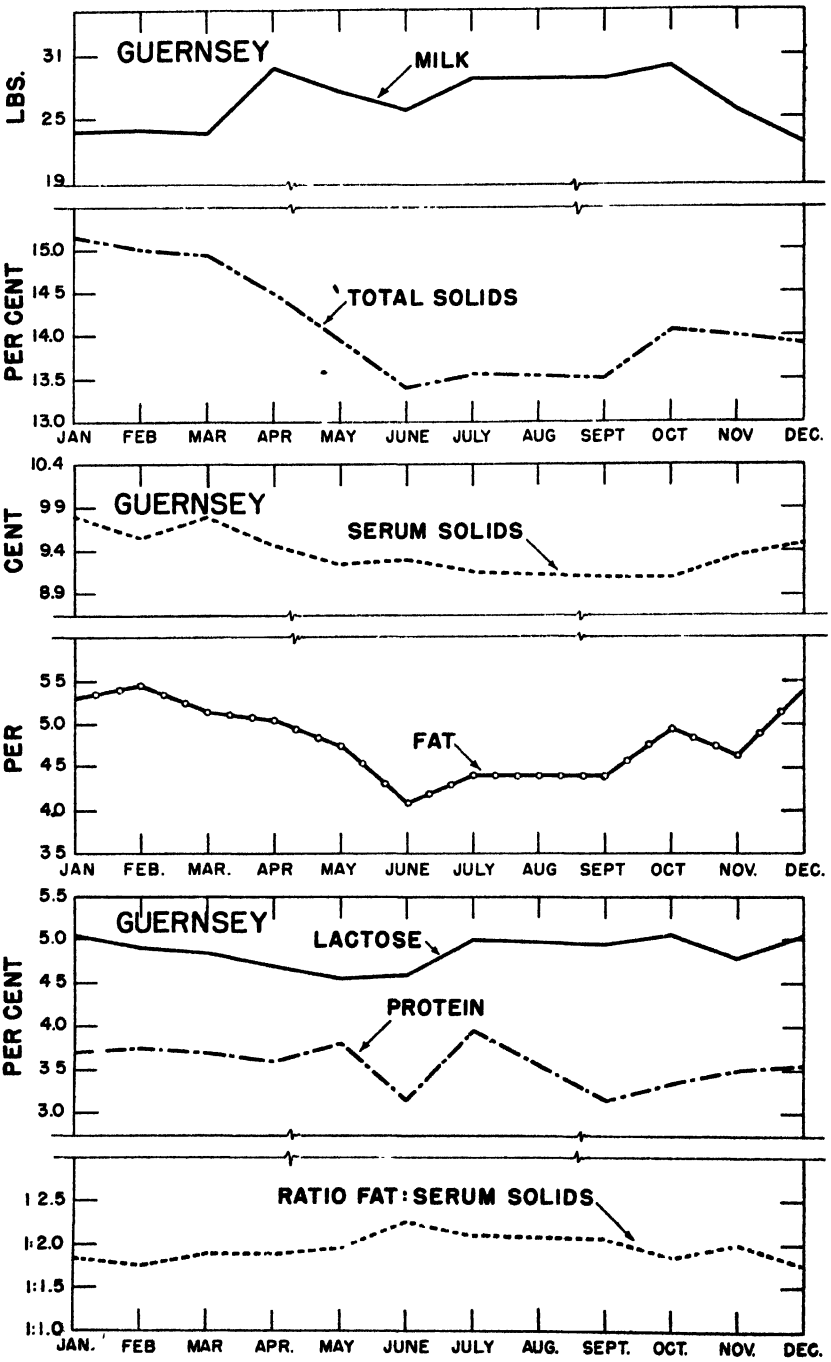

FIG. 2. Variations in constituents of milk from Guernsey cows by calendar months.

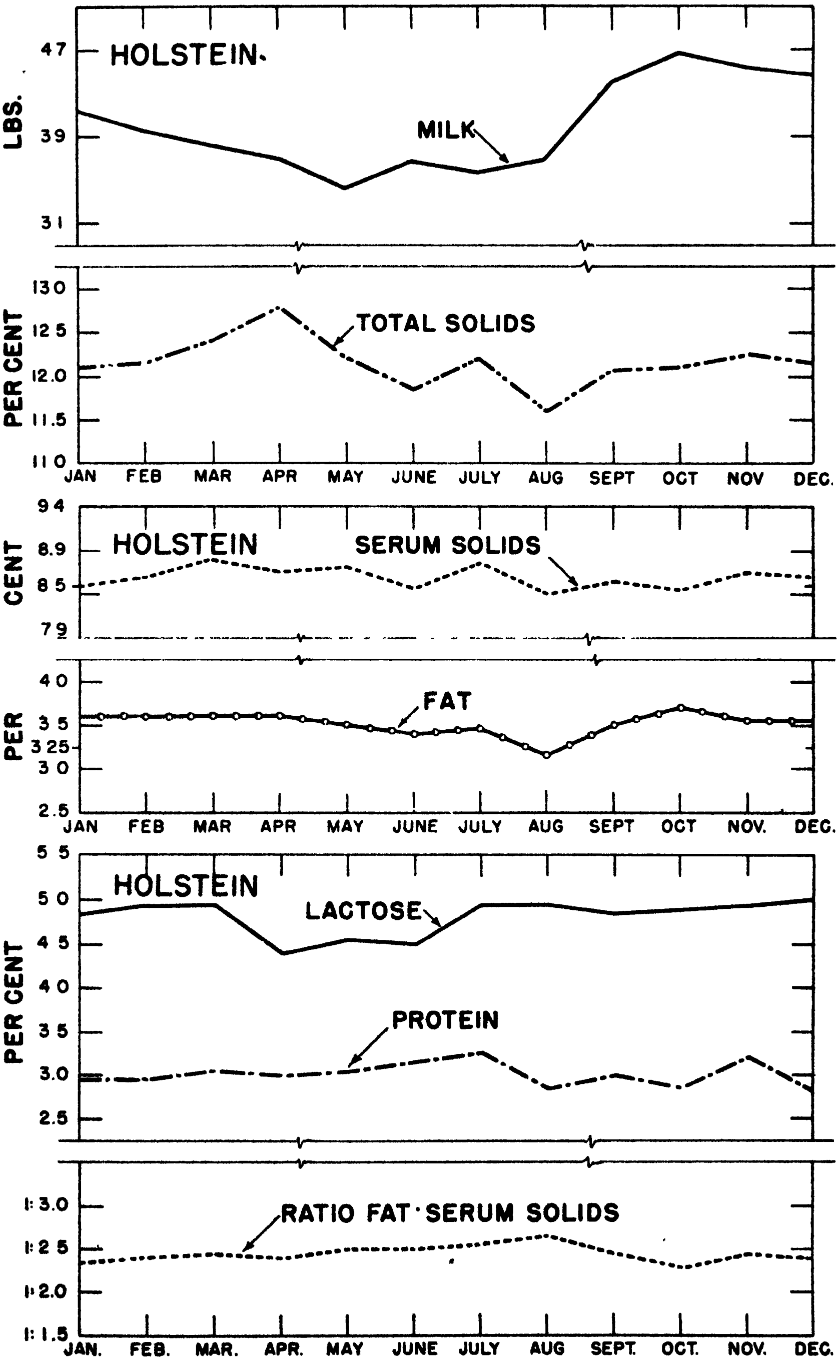

FIG. 3. Variations in constituents of milk from Holstein cows by calendar months.

Weighted averages were calculated by dividing the pounds of each constituent by the production. The maximum, minimum, and average values of the combined data are shown in table 1.

TABLE 2

Samples of milk with abnormal relationship between fat and serum solids

% Fat	% Serum solids				
	Determined analytically	Skim milk*	Normal milk†	Normal skim milk*	% Fat for serum solids‡
Jersey					
7.8	9.33	10.12	10.19	11.05	5.7
7.4	9.66	10.43	10.03	10.83	6.5
7.2	9.32	10.04	9.95	10.72	5.6
6.9	9.15	9.83	9.83	10.56	5.2
6.7	8.88	9.52	9.75	10.45	4.5
6.3	7.85	8.38	9.59	10.23	2.0
Av. 7.05	9.03	9.71	9.89	10.64	4.92
Guernsey					
5.9	8.00	8.50	9.43	10.02	2.3
5.5	8.86	9.50	9.27	9.81	4.5
5.0	8.74	9.20	9.07	9.55	4.2
4.6	8.13	8.52	8.91	9.34	2.7
4.2	8.26	8.62	8.75	9.13	3.0
Av. 5.04	8.40	8.87	9.09	9.57	3.34
Holstein					
7.8	9.36	10.15	10.19	11.05	5.8
6.1	7.39	7.87	9.51	10.13	0.8
Av. 6.95	8.38	9.01	9.85	10.59	3.30

* Serum solids of skim milk calculated by method of Sommer (12).

† Serum solids values of normal milk calculated by method of Jacobson (6).

‡ Normal percentage of fat for serum solids in the sample calculated by method of Jacobson (6).

DISCUSSION

The values in table 1 show the most widely variable constituent of milk to be the butterfat, followed in order by protein, total solids, lactose, and serum solids. The maximum percentage of butterfat is 3.0 times the minimum value; the corresponding values for protein, total solids, lactose, and serum solids are 1.74, 1.63, 1.56, and 1.54, respectively. The maximum and minimum values in table 1 include samples which were abnormally high in fat and abnormally low in serum solids. Two samples of Jersey milk had abnormally high fat tests of 7.8 and 7.4 per cent, the remaining samples testing from 3.4 to 7.2 per cent fat. One Jersey sample contained only 7.38 per cent serum solids, the next lowest test for this breed being 8.24 per cent. Except for one sample with a value of 7.38 per cent, the Guernsey samples varied from 8.00 to 11.54 per cent serum solids. Two samples of Holstein milk tested 7.8 and 6.1 per cent fat, the other 205 samples testing 2.6 to 4.8 per cent fat. The two lowest serum solids values for Holstein milk were 7.39 and 7.72 per cent.

Comparison of these values with results of other investigators shows slightly lower total solids averages for the three breeds studied. The average butterfat percentages of Jersey and Holstein milk were essentially the same as the values given by Overman *et al.* (7), and 1.31 per cent below the value reported by Sherman (10) on monthly samples over a 5-year period. The Guernsey fat content was found, however, to be lower than that reported by other workers (3, 7). The lactose and protein contents of Guernsey milk and the lactose values of Holstein milk were in agreement with these other investigators, while the protein content of Holstein milk and the lactose and protein contents of Jersey milk were lower. The fact that many of these values are lower than is reported in the literature might be attributed, in part, to the higher mean annual temperature of the Tucson area (11) as contrasted with other localities from which data have been collected.

The four individuals which represent one cow family have lowered the average value for each constituent of the milk of the Jersey breed in this study. The data on the milk from these cows show the milk to be 0.28 per cent lower in total solids and 0.31 per cent lower in lactose. The serum solids, fat, and protein were 0.22, 0.05, and 0.07 per cent lower, respectively. The results obtained from the data on the milk from the remaining seven Jersey cows are in agreement with results of Overman *et al.* (7).

As indicated in table 1, the minimum value for serum solids of certain individual cows of each of the three breeds was lower than the existing legal requirement of 8.5 per cent for Arizona. The averages for the Jersey, Guernsey, and Holstein breeds were 9.45, 9.39, and 8.59 per cent, respectively. The data show the serum solids values to be exceedingly low in proportion to fat in some of the samples. Four samples (2.5 per cent) of Jersey milk, three samples (2.16 per cent) of Guernsey milk, and 97 samples (47.5 per cent) of the milk from Holstein cows were below the legal minimum. Each of the Holstein cows produced milk low in serum solids at some time during the experiment; samples below 8.50 per cent serum solids varied from 9.0 to 87.5 per cent of those from each cow. Nine Holstein cows sired by the same bull had an average ratio of fat to serum solids of 1:2.40 as compared to 1:2.57 for the three other cows of this breed. Three of the group of nine and one of the group of three cows had average serum solids values below the legal requirement. These results are in agreement with work of Caulfield *et al.* (1), who reported that 9 of 25 Holstein cows produced milk which averaged less than 8.5 per cent serum solids.

The fat tests of the milk from the twelve Holstein cows show 47 samples (22.7 per cent) were less than 3.25 per cent. The nine related cows had 20 tests (9.7 per cent) below 3.25 per cent as compared to 27 (13.0 per cent) for the other group. One sample of Guernsey milk contained less than 3.25 per cent fat. All samples of Jersey milk exceeded the minimum fat requirement.

The data in table 2 show six samples (1.9 per cent) of milk from individual Jersey cows to have abnormal relationships between fat and serum solids. Five samples (3.6 per cent) of Guernsey milk were abnormally low in serum solids, with values of 8.00 to 8.86 per cent for milk containing 4.2 to 5.9 per cent fat. Two samples (0.1 per cent) of milk from Holstein cows had abnormal fat tests of 6.1 and 7.8 per cent with serum solids values of 7.39 and 9.36 per cent.

Figure 3 also shows the monthly average serum solids content of Holstein milk was less than 8.50 per cent in June, August, and October, with slightly higher values in the intervening months. Experimental evidence on the effect of environmental temperatures on the percentage of fat in cow's milk has been presented by numerous investigators (5, 8, 12, 13). Turner (13) reported an increase of almost 0.2 per cent in the fat content of milk with each decrease of 10° F. in environmental temperature between 30 and 70° F. The data reported herein indicate that the higher environmental temperatures of spring and summer are responsible, in general, for the lower fat content of the Jersey milk. The mean maximum temperatures for spring, summer, fall, and winter seasons were 82.6, 99.3, 84.8, and 66.9° F., respectively (table 3). The fat tests of milk from Jersey cows during these seasons were 4.95, 4.84, 5.09, and 5.51 per cent. These fat tests represent ten lactations begun in the spring and summer when the mean maximum temperature was 91.0° F. and ten begun in the fall and winter period when the mean maximum temperature was 75.9° F.

TABLE 3

The number of lactations begun by season for each breed and mean maximum temperatures during experimental period

Season	Mean maximum temp.	Jersey	Guernsey	Holstein
	(°*F.*)			
Spring	82.6	8	5	3
Summer	99.3	2	8	11
Fall	84.8	7	4	8
Winter	66.9	3	2	3

The fat tests of the milk from Guernsey cows for the same periods were 5.06, 4.26, 4.72, and 5.49 per cent, respectively. Thirteen lactations were begun in spring and summer and six in the fall and winter months. The fat test for the summer period was lowest, and milk production on the "fat-corrected-milk" basis also was lowest then. These results indicate that the higher environmental temperature had a greater influence on the fat test of Guernsey milk than did the advancing stage of lactation.

The Holstein milk contained 3.49, 3.42, 3.57, and 3.61 per cent fat in the respective seasons. Inasmuch as fourteen lactations were nearing completion in early spring and summer and eleven lactations begun in the cooler months were approximately at mid-point, the average fat test was consistently lower during the warmer months.

The data presented in table 4 show the average percentage of fat in the milk from the cows of the three breeds with each 10° variation in mean maximum temperature between 60 and 109° F. The average for all samples shows a decrease of 0.1 per cent fat for each 10° rise in mean maximum temperature. These data are in agreement with results of Turner (13), who reported an increase of 0.2 per cent fat for each 10° decrease in environmental temperature between 30 and 70° F. The differences in climatic conditions have reflected less effect on the fat content of the milk at the higher temperatures in Arizona. The great diurnal temperature range of this section (11), accompanied by lower humidity, produces high daytime temperatures and considerably lower temperatures at night. Daily temperatures may vary as much as 45 to 65° F., with an average variation of 30°.

TABLE 4

Average fat tests by range of mean maximum temperatures

Range (° F.) Av. (° F.)	60–69 63.6		70–79 74.3		80–89 85.7		90–99 94.3		100–109 103.7	
	No. samples	Av. % fat	No. samples	Av. % fat	No. samples	Av. % fat	No. samples	Av. % fat	No. samples	Av. % fat
Jersey	28	5.12	48	4.85	36	4.90	27	4.98	21	4.56
Guernsey	26	5.37	38	5.37	39	4.94	19	4.47	17	4.75
Holstein	43	3.61	58	3.54	46	3.36	32	3.43	23	3.57
Av.		4.55		4.46		4.35		4.27		4.27

SUMMARY

(1) In general, a corresponding rise was noted in the total solids, serum solids, and protein when the butterfat content increased, except that in some cases samples were abnormally high in fat and low in serum solids.

(2) An increase in the protein content was accompanied by increased butterfat, total solids, and serum solids, and by a decrease in the ratio of fat to serum solids.

(3) An inverse relationship existed between milk production and total solids, serum solids, and butterfat percentages of the milk.

(4) In general, an inverse relationship was present between the lactose and the protein percentage of the milk.

(5) The total solids, butterfat, and serum solids content of the milk was higher in winter and lower in summer. The difference presumably is due mainly to the higher environmental temperatures of the summer months.

(6) The lower butterfat test of the Guernsey milk is due primarily to the higher environmental temperature. Milk from Guernsey cows had the greatest variation in fat test by season of the three breeds studied.

(7) Environmental temperatures appear to exert a greater influence on milk and butterfat production than does the advancing stage of lactation.

(8) The percentage of samples of milk falling below the minimum state requirement for serum solids and fat, respectively, were: Holstein, 47.5 and 22.7; Guernsey, 2.2 and 0.6; Jersey, 2.5 and 0.

(9) Using averages for all animals as the basis of calculation, the butterfat content of milk decreased 0.1 per cent for each 10° rise in mean maximum temperature between 65 and 105° F. under Arizona conditions.

(10) A Jersey cow family was found to produce milk 0.31 per cent lower in lactose than normal for the Jersey breed.

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VARIATION IN THE CONSTITUENTS OF MILK UNDER ARIZONA CONDITIONS. II. INFLUENCE OF THE MONTH OF LACTATION IN COWS OF DIFFERENT BREEDS

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A definite relationship usually is considered to exist between the stage of lactation and the composition of milk. This study is concerned with such variations under Arizona conditions. Work has been reported which would indicate that environmental temperature exerts a greater influence than advancing lactation upon the constituents of milk.

REVIEW OF LITERATURE

White and Judkins (9) studied the variations in serum solids and fat in milk of cows freshening in each of the 12 months of the year. They reported that on 87 lactations of 80 cows over 7.5 years, the lowest serum solids content of the milk was found in June and July and the highest values during the winter months, irrespective of the time at which the lactations began. The butterfat percentages usually decreased during the second month and increased to the end of the lactation except when the lactation ended in summer, in which case the fat content was not significantly higher. Ragsdale and Turner (6) also found, in the study of 4,045 records, that the butterfat decreased in the second month. In some cases this decline continued for at least 3 months and then the per cent of butterfat gradually increased. These investigators concluded that the stage of lactation influences the fat content of the milk and the variations are independent of the season of the year. Turner *et al.* (7) concluded from the study of 3,215 lactations that Guernsey cows produce 94 per cent of the previous month's milk production through the entire period of decline of milk secretion. Van Slyke (8) noted similar butterfat trends and reported a decrease in protein in the second month of the lactation; these two constituents then increased in the following months. The data on 293 lactations presented by Becker and Arnold (2) show that the butterfat content of Jersey milk was lowest (4.59 per cent) in the second month of the lactation and increased to 5.56 per cent in the final month. These authors also report that the average butterfat level varied by calendar month in Florida in the years 1917 to 1932 from 4.76 per cent in August to 5.67 per cent in September. Wylie (11) reported that of 326 Jersey cows freshening in January, the initial fat

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test of 4.89 per cent increased slightly to the sixth month and rose toward the end of the lactation. The milk of 125 cows freshening in July showed a higher fat content in January. All other lactations showed the highest fat content of the milk at the end of the lactation. These investigators concluded that the stage of lactation exerted greater influence than did the season of the year on the fat content of the milk.

Bartlett (1) reported on 294 complete lactations, each of 8 months or more, emphasizing the influence of the age of the cow upon variations of milk constituents. Cows in their first lactation showed an average variation in serum solids of 9.46 to 9.81 per cent as compared to 9.24 to 9.65 per cent in the second lactations; the serum solids of mature cows was reported to be 9.18 to 9.62 per cent. Corresponding figures for barren cows were slightly lower and even declined toward the close of the lactation. The increase of serum solids in advanced lactation became less pronounced as the cow aged; at the ninth lactation serum solids were only 94.5 per cent of that of the first lactation. Woodward (10) plotted milk production and fat tests from lactation records of 15,442 cows in twelve states. He noted that the peak of milk production was reached in the second month. The butterfat percentage was lowest in the third month, after which there was a steady increase.

EXPERIMENTAL

The cows selected for this study were eleven purebred Jersey, eleven Guernsey, and twelve Holstein cows of the University of Arizona Herd. The lactations studied began in the various months throughout the 30-month experimental period. Analyses were made monthly on representative samples of milk from individual cows. The data collected represent seven to ten monthly tests during each lactation. The lactose and protein values represent complete lactations of four of the eleven Jersey, five of eleven Guernsey, and nine of twelve Holstein cows. Weighted averages were used in the presentation of the data and are computed by dividing the total pounds of each constituent by the total pounds of milk produced.

The butterfat content of the milk was determined by the Babcock test, total solids by the Mojonnier method, and serum solids by calculation of difference. The polariscope was used to analyze for lactose. The Kjeldahl method was used to determine total nitrogen; the total nitrogen value was multiplied by the factor 6.40 to give the percentage of protein.

The average variations of each constituent of milk by month of lactation for the Jersey, Guernsey, and Holstein cows are presented in figures 1 to 3. The data represent 64 complete lactations for the 34 cows of the three breeds. Twenty-five cows were in their first three lactations and the remaining nine in their fourth to sixth lactations at the beginning of the experiment. Data were plotted to indicate the average day of the month of the lactation on which samples were obtained for analyses. Table 1 shows com-

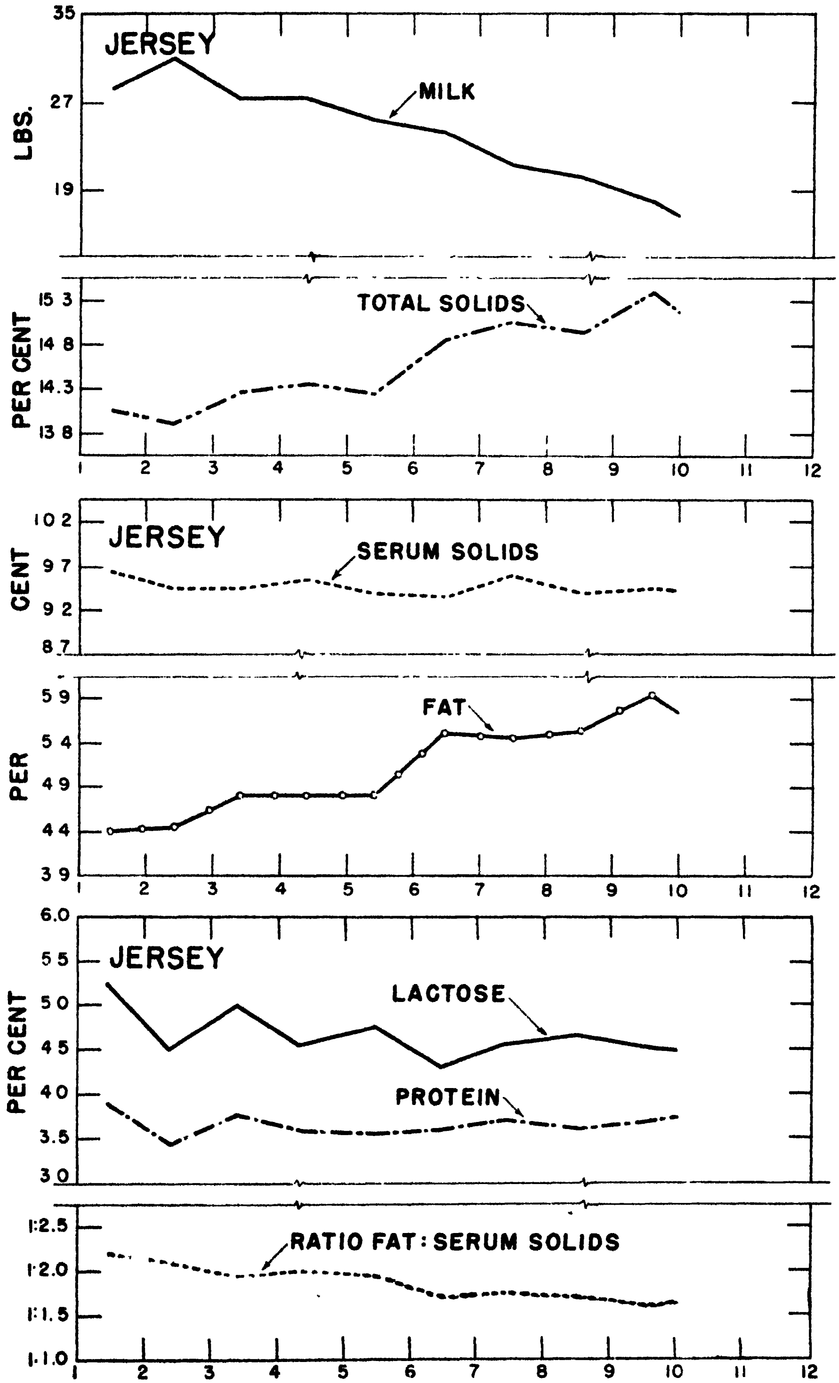

FIG. 1. Variations in constituents of milk from Jersey cows by month of lactation.

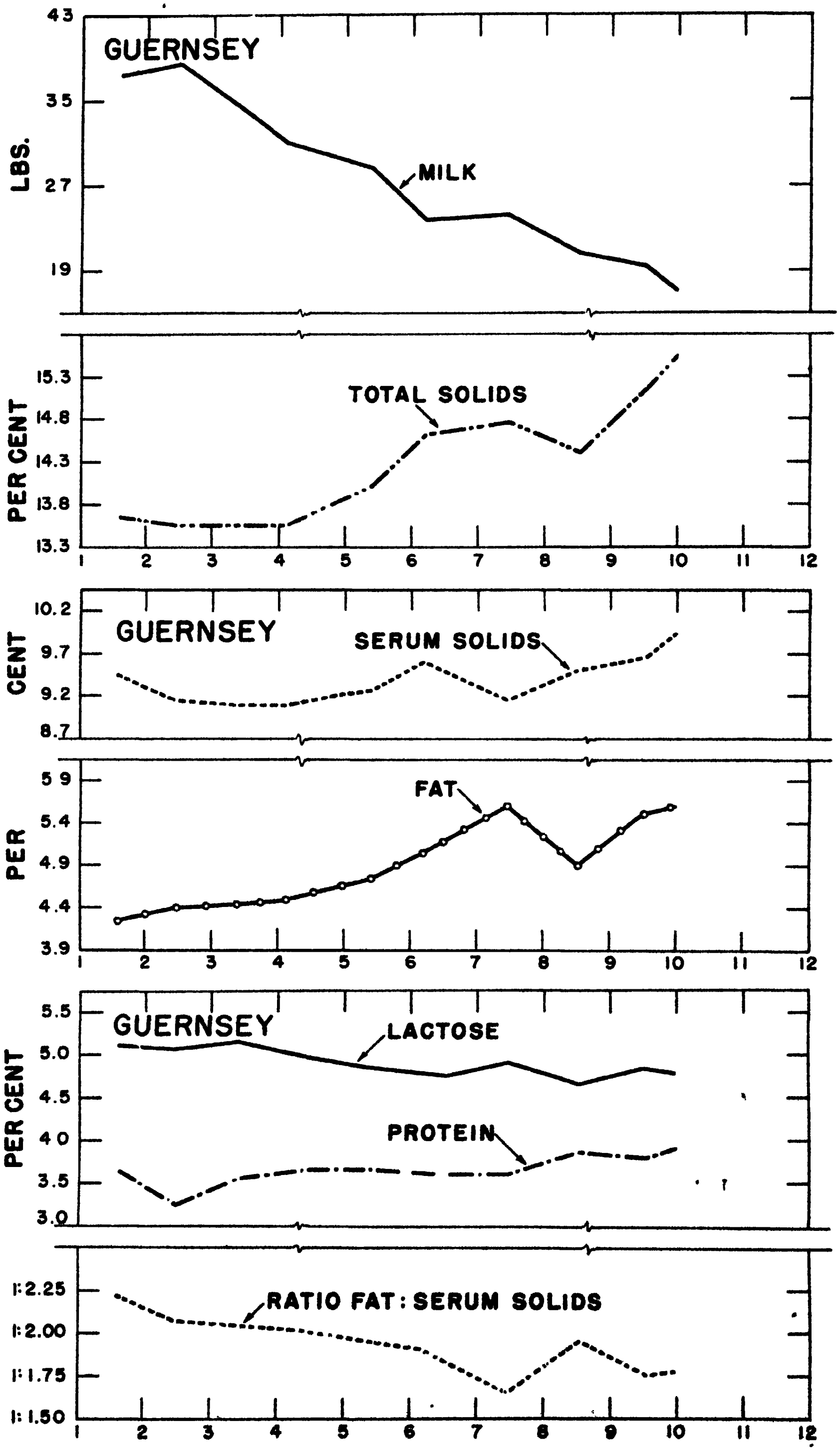

FIG. 2. Variations in constituents of milk from Guernsey cows by month of lactation.

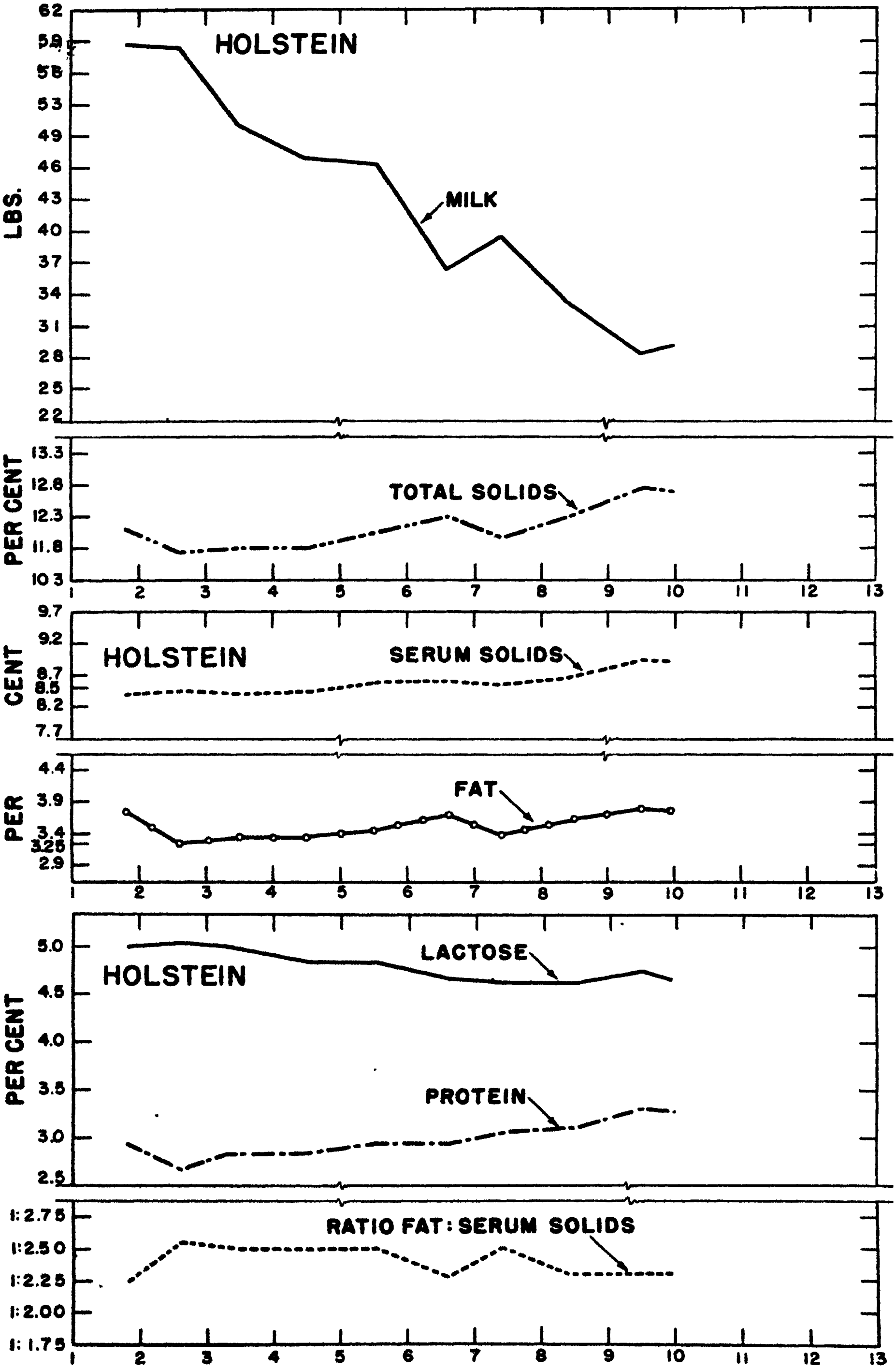

FIG. 3. Variations in constituents of milk from Holstein cows by month of lactation.

parisons in the results of this study with results obtained by Overman *et al.* (5) at the Illinois Station.

TABLE 1

Comparison by breeds of data on milk composition as obtained in this study and by Overman et al. (5) at the Illinois Agricultural Experiment Station

Breed	Jersey		Guernsey		Holstein	
No. of samples	Ariz. 103	Ill. 199	Ariz. 100	Ill. 321	Ariz. 159	Ill. 268
Constituent	(%)	(%)	(%)	(%)	(%)	(%)
Total solids	14.62	14.6941	14.24	14.8562	12.11	12.2667
Serum solids	9.45	9.5105	9.38	9.6698	8.58	8.8615
Fat	5.16	5.1836	4.85	5.1865	3.53	3.4050
Protein	3.68	3.8629	3.56	4.0152	3.00	3.2987
Lactose	4.74	4.9447	4.86	4.9126	4.83	4.8875

DISCUSSION

The data presented in figure 1 show, in part, the daily milk production of the Jersey cows. The initial rise in production represents an average increase of 2.65 lbs. (8.52 per cent) of milk per day in the second month of the lactation. A gradual decrease of 44.96 per cent from the third month to the end of the lactation was accompanied by a decrease in the lactose content of 10.42 per cent and increases in the fat and total solids content of the milk of 12.11 and 7.54 per cent, respectively. Figure 1 also shows that the serum solids values did not rise with the corresponding increase in fat in the last 4 months. This is shown also by the ratio of fat to serum solids. Data for each cow show the average fat test ranges from 4.4 to 6.1 per cent, serum solids from 9.0 to 9.6 per cent, and lactose from 4.4 to 5.3 per cent.

All of the values obtained on the constituents of Jersey milk were lower than values reported by Overman *et al.* (5). The average lactose value of 4.74 per cent is 0.20 per cent lower. The four Jersey cows producing milk low in lactose and also in the other milk constituents were sired by the same bull. The protein value of 3.68 per cent was 0.18 per cent lower. Since the fat content was only 0.02 per cent lower, the lower lactose and protein values were mainly responsible for lower total solids and serum solids values.

The total solids, fat, and protein content of milk from Jersey cows decreased 1.00, 2.01, and 11.57 per cent, respectively, from the first to the second month and increased 6.40, 26.51, and 8.99 per cent from the second to the tenth month of the lactation. The serum solids varied only 3.10 per cent between the highest and lowest value.

Figure 2 shows that in Guernsey milk, the percentages of total solids, serum solids, fat and protein increased throughout the lactation with the exception of the eighth month, in which only the protein and serum solids values increased over the previous month. Eight lactations were begun in the spring and summer months. The two previous months of these lactations were in the cooler months and showed a marked increase in the total solids, which may be a result of lower environmental temperature. Also, two of the ten samples obtained from ten cows in their seventh month of lactation were considered abnormally low in serum solids, with values of 8.00 and 8.92 per cent for milk containing 5.9 and 5.7 per cent fat, respectively. The average serum solids value of the remaining eight cows was 9.45 per cent, only slightly lower than the values of 9.58 and 9.51 per cent for the sixth and eighth months of lactation. Fifty-one per cent of the samples had lactose and protein determinations, and this group had slightly higher total solids, serum solids, and fat values.

The milk from the Guernsey cows decreased in total solids and serum solids from the first to the second month by 0.3 and 3.0 per cent, respectively. The fat and protein content increased 4.1 and 10.4 per cent, respectively, from the first to the second month, and increased 21.8 and 14.7 from the second to the tenth month. The total solids and serum solids increased 14.1 and 9.9 per cent in the same period.

Figure 3 shows the variations in constituents of milk from Holstein cows throughout the lactation. The milk production was highest in the first month of lactation, with an average of 58.9 lbs. per day In the second month 58.1 lbs. were produced, and the milk production decreased to the end of the lactation. The corresponding increase in total solids was from 11.7 per cent in the second month to 12.8 and 12.6 per cent in the ninth and tenth months. The serum solids increased only 6.0 per cent during the entire lactation. The lactose was highest in the first 3 months, with values of 5.02, 5.04, and 5.01 per cent, respectively, and decreased to 4.54 per cent in the tenth month. The protein increased steadily from 2.66 per cent in the second month to 3.30 per cent in the ninth month.

The average fat content of Holstein milk in the second month was 3.27 per cent, only slightly above the legal minimum. The fat increased to 3.36 per cent in the fourth month, but the milk was below the minimum in serum solids, with values of 8.42, 8.47, 8.42, and 8.44 per cent, respectively, in each of the first 4 months. The serum solids increased gradually to 8.96 and 8.89 per cent in the ninth and tenth months, respectively, with fat tests of 3.81 and 3.76 per cent in the same months. In the first 4 months of the lactation 62.0 per cent of the samples were below 8.50 per cent serum solids as compared to 32.5 per cent in the 6 remaining months. Thirty-four per cent of the samples were below 3.25 per cent fat in the former period and 18.7 per cent in the latter period.

The milk from Holstein cows showed decreases of 3.69, 13.26, and 9.52 per cent in the total solids, fat, and protein content from the first to the second month of lactation. The serum solids increased 0.59 per cent in that time. The production from the second to tenth month showed increases of 7.12, 4.72, 13.01, and 17.65 per cent in total solids, serum solids, fat, and protein, respectively.

The decline in milk production throughout the lactation was approximately the same for the Jerseys and Holsteins and slightly greater for the Guernseys, as shown by decreases of 51.50, 51.94, and 59.49 per cent, respectively. Milk from Jersey cows averaged 14.65 per cent less lactose at the end of the lactation, as compared to 9.88 and 9.92 per cent for the Guernsey and Holstein cows. This difference was due, in part, to the four related Jersey cows producing milk consistently low in lactose. The increase in serum solids was 10.33 for Guernsey milk, 6.03 for Holstein milk, and 2.19 per cent for Jersey milk. The milk of the higher testing breeds increased more in fat content during the lactation (Jersey, 26.5; Guernsey, 25.0; and Holstein, 14.2 per cent). The protein content of milk from Holstein cows increased 19.4 per cent as compared to 11.6 for Jersey and 18.3 for Guernsey cows.

Milk of any particular cow abnormally high in fat is likely to be abnormally low in serum solids, as was shown in the preceding paper of this series (3). The data on milk from Holstein cows show nine samples with an average serum solids value of 8.1 per cent in the eighth month and with 3.6 per cent fat. Milk containing 3.6 per cent fat should contain 8.5 per cent serum solids as calculated by the method of Jacobson (4). The ratio of fat to serum solids will vary with individuals even though the fat percentage remains the same. The greatest variation was from 8.96 to 11.25 per cent serum solids for Jersey milk containing 3.8 per cent fat. The maximum variations for Holstein and Guernsey milk were 2.23 and 1.90 per cent for milk with 3.8 and 4.6 per cent fat, respectively.

SUMMARY

The results of analyses of 362 samples of milk were compiled and presented graphically by breeds and compared with results obtained by the Illinois Station. The samples were taken 1 day each month throughout the lactation periods and results were compiled by month of lactation.

(1) The data show steady increases in total solids, serum solids, fat, and protein values after the second month of the lactations, and a slight decrease in the values of the constituents of Guernsey milk during the eighth month. A large percentage of the cows were in the eighth month of their lactation during the spring and summer months.

(2) Milk produced under Arizona conditions averaged lower in all constituents than milk produced in Illinois, except for the fat content of milk from the Holstein breed.

(3) The serum solids of Jersey milk showed less variation than that of the milk of the other breeds studied, as shown by variation of 0.35 per cent

throughout the lactation as compared to 1.08 per cent and 0.65 per cent variations for the Guernsey and Holstein milk, respectively.

(4) The total solids, fat, and protein of Holstein milk decreased 3.69, 13.26, and 9.52 per cent from the first to the second month and then increased 7.12, 13.03, and 17.65 per cent, respectively, to the end of the lactation. The milk from Jersey and Guernsey cows showed 2.01 and 4.08 per cent increase in fat from the first to second month. The milk increased in fat 26.51 and 21.81 per cent, respectively, in the remainder of the lactation. The milk from the Jersey and Guernsey cows decreased 1.00 and 0.59 per cent in total solids in the second month and increased 6.40 and 14.09 per cent, respectively, from the second month to the end of the lactation. The protein content of the Jersey milk decreased 11.57 per cent in the first period, while Guernsey milk increased 10.44 per cent. The increases for the latter period were 8.99 and 14.66 per cent for Jersey and Guernsey milk, respectively.

(5) The ratio of the fat to serum solids shows that as the lactation advances the fat content is higher in proportion to the serum solids.

(6) The ratio of fat to serum solids will vary with individual cows even though the fat test is the same.

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VARIATION IN THE CONSTITUENTS OF MILK UNDER ARIZONA CONDITIONS. III. VARIATION IN MILK FROM JERSEY, GUERNSEY, HOLSTEIN, AND MIXED HERDS

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Considerable daily variation is known to occur in the composition of milk of individual cows of each breed. The milk, however, usually is sold not from individual cows but from herds of cows. Individual variations among cows, therefore, are of less significance in mixed milk. Herds producing milk having low fat and serum solids tests can be changed to include other animals of the same breed or other breeds which produce milk higher in fat and serum solids. This study is concerned with the variations in the composition of the milk from herds of Jerseys, Guernseys, Holsteins, and mixtures of two or more breeds and from herds maintained in good physical condition as compared with marginal herds.

REVIEW OF LITERATURE

Lythgoe (6) reported 45 analyses of samples from herds in which the total solids, fat, serum solids, and protein were higher in winter and lower in summer. Jacobson (4) reported on the analyses of over one hundred thousand samples of mixed milk and found that serum solids increased consistently 0.04 per cent with each 0.10 per cent increase in fat. This investigator also observed that milks containing 3.2 to 4.8 per cent fat showed considerably lower serum solids in winter than did the milks containing 4.9 to 5.7 per cent fat. July, August, and September were characterized by a reduction in serum solids values.

Little (5) studied the chloride content of milk from 24 cows at various stages of lactation. He reported that during the first 4 months of lactation, 90 per cent of the chloride values were under 0.14 per cent, as compared to 13.4 per cent under that value after 8 months or more of lactation. Sharp and Struble (8) conducted monthly chloride titrations on thirteen Jersey, eight Guernsey, and fifteen Holstein cows. These investigators noted a rapid decline in chlorides during the first few days, a slight increase during the first 60 per cent of the lactation, and a marked increase during the final 10 per cent of the lactation period.

Sharp and Hart (7) reported that, on results of ten samples, lactose had very little significance in the variation of specific gravity. Gaines (3)

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studied the milk of 52 cows of various ages, weights, and breeds, and concluded that the secretion of fat and protein appear intimately related. The ratio of fat to protein reported by this investigator was 1 : 0.812 ± 0.010, and this relationship was applicable to a fat range in the milk from 0.36 to 22.46 per cent.

EXPERIMENTAL

The milk samples analyzed in this study were obtained from two purebred Jersey, one purebred Guernsey, three purebred Holstein, and five mixed herds, each of 30 cows or more. Representative samples were taken monthly from the mixed milk of each herd.

The butterfat content was determined by the Babcock method, total solids by the Mojonnier method, and serum solids by calculation of difference. The polariscope was used to analyze for lactose. Total nitrogen percentage was obtained by the Kjeldahl method; the total nitrogen then was multiplied by the factor 6.40 to give the percentage of protein. The specific gravity was determined by use of a hydrometer. The chloride determinations were made by direct titration with tenth normal silver nitrate in the presence of potassium chromate.

The data collected on the four groups of herds are presented in figures 1 to 4, inclusive. The data represent 48 tests on the milk from Jersey herds, 20 from Guernsey herds, 50 from Holstein herds, and 120 from the mixed herds. The tests were made over a period of 2 years. The maximum, minimum, and average values for the constituents of milk are presented by

TABLE 1

Maximum, minimum, and average per cent of different constituents of milk for the four groups of herds

	Total solids	Serum solids	Fat	Lactose	Protein	Chlorides	Ratio serum solids to fat	Specific gravity
	(%)	(%)	(%)	(%)	(%)	(%)		
Jersey								
Max.	15.4	10.1	5.7	5.4	3.9	0.13	2.41	1.0355
Min.	13.1	8.1	4.1	4.4	2.9	0.06	1.41	1.0310
Av.	14.2	9.3	4.9	4.8	3.5	0.11	1.89	1.0348
Guernsey								
Max.	14.3	9.7	4.8	5.2	3.6	0.21	2.36	1.0360
Min.	12.2	8.2	3.9	4.4	3.1	0.11	1.89	1.0305
Av.	13.6	9.2	4.4	4.8	3.3	0.13	2.11	1.0341
Holstein								
Max.	12.9	9.1	4.4	5.3	3.2	0.19	2.79	1.0345
Min.	11.1	7.7	3.0	4.1	2.2	0.13	1.80	1.0305
Av.	12.0	8.5	3.5	4.7	2.9	0.15	2.43	1.0323
Mixed								
Max.	13.7	9.2	5.0	5.4	3.6	0.16	2.65	1.0355
Min.	11.8	8.0	3.3	4.2	2.4	0.09	1.69	1.0310
Av.	12.6	8.8	3.8	4.6	3.2	0.13	2.28	1.0330

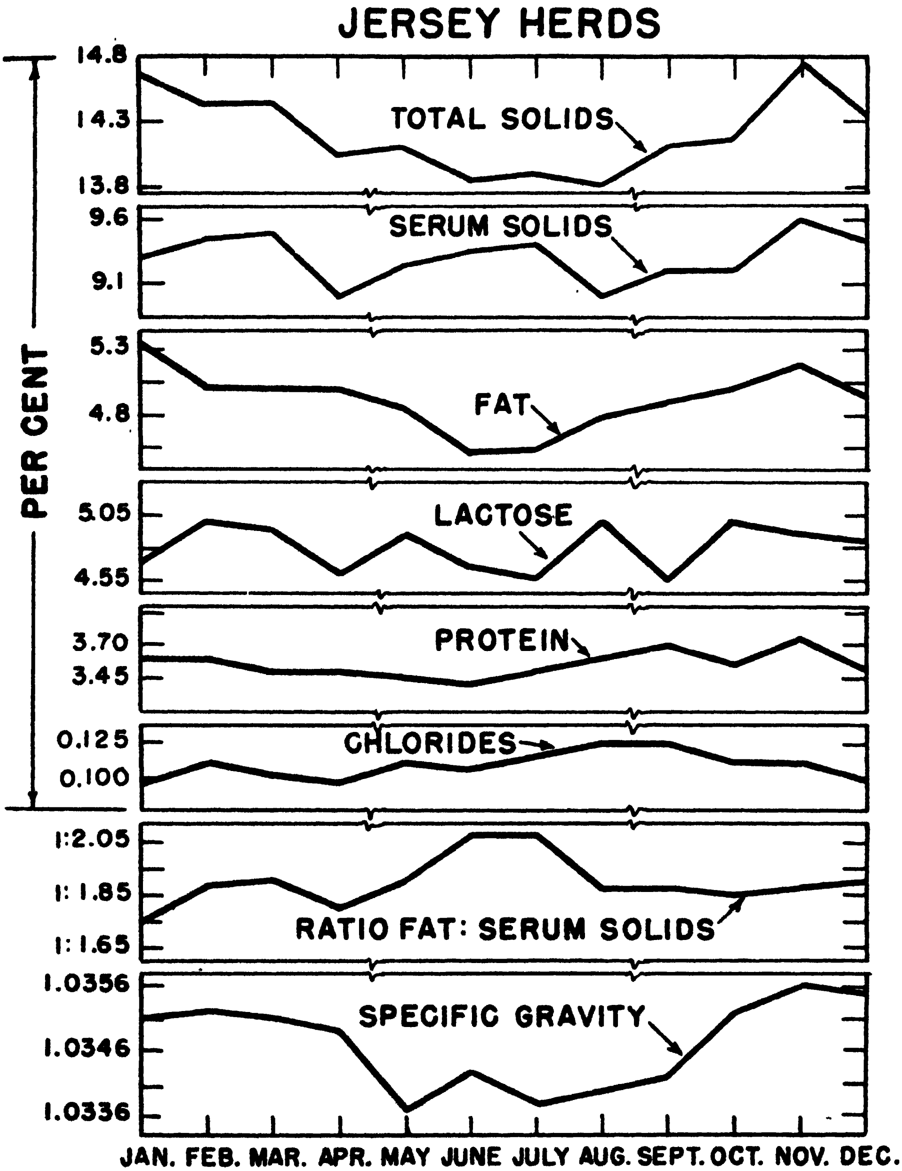

FIG. 1. Variations in constituents of milk from Jersey herds by calendar months.

groups in table 1. Table 2 represents the percentage variation in composition of milk from winter to summer.

Figures 1 to 4 show that the total solids, serum-solids, fat, protein, and lactose content of milk from each group of herds were lowest in summer and highest in winter. Table 2 shows the percentage decrease in total solids,

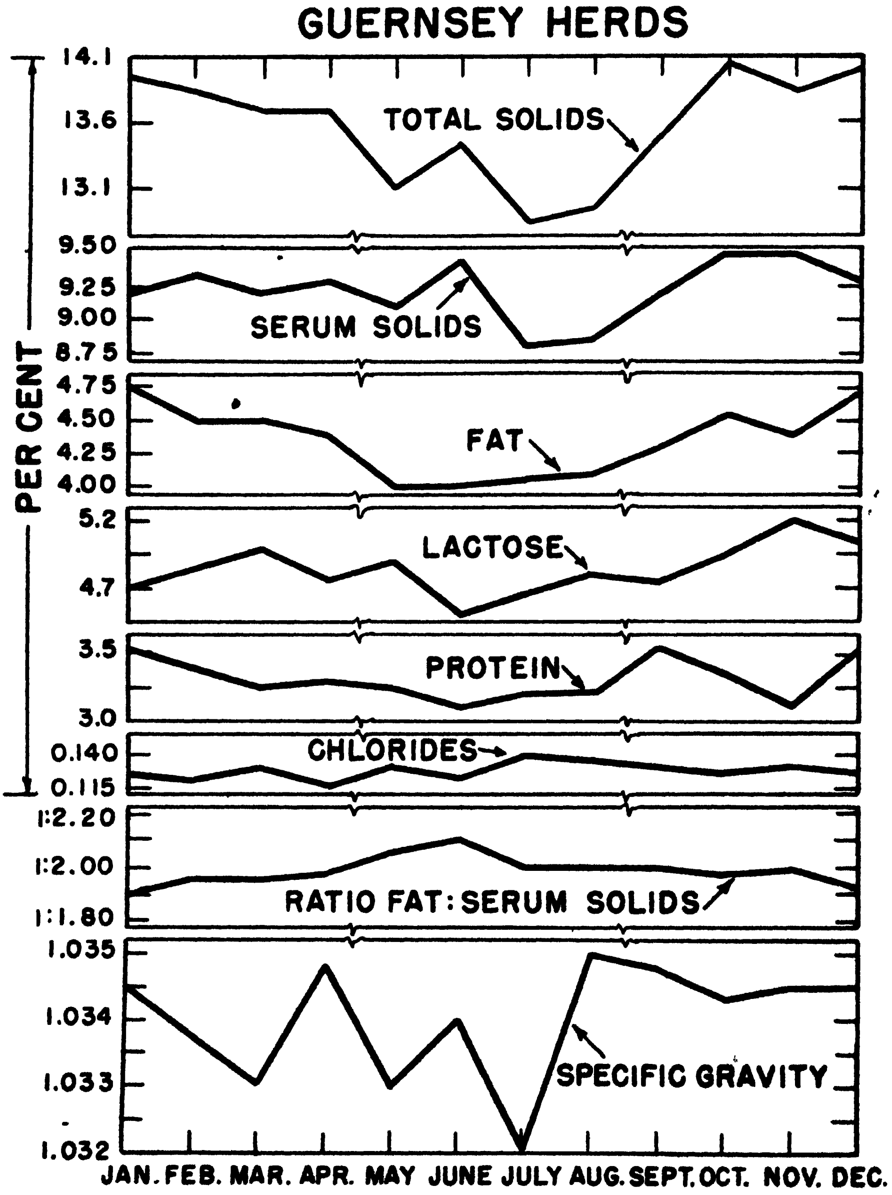

FIG. 2. *Variations in constituents of milk from Guernsey herds by calendar months.*

serum solids, fat, lactose, and protein and the increase in the chloride content of the milk from each of the four groups. The specific gravity of milk from Jersey, Guernsey, Holstein, and mixed herds averaged 1.035, 1.034, 1.032, and 1.034, respectively, in winter and 1.034, 1.034, 1.032, and 1.033, respectively, in summer.

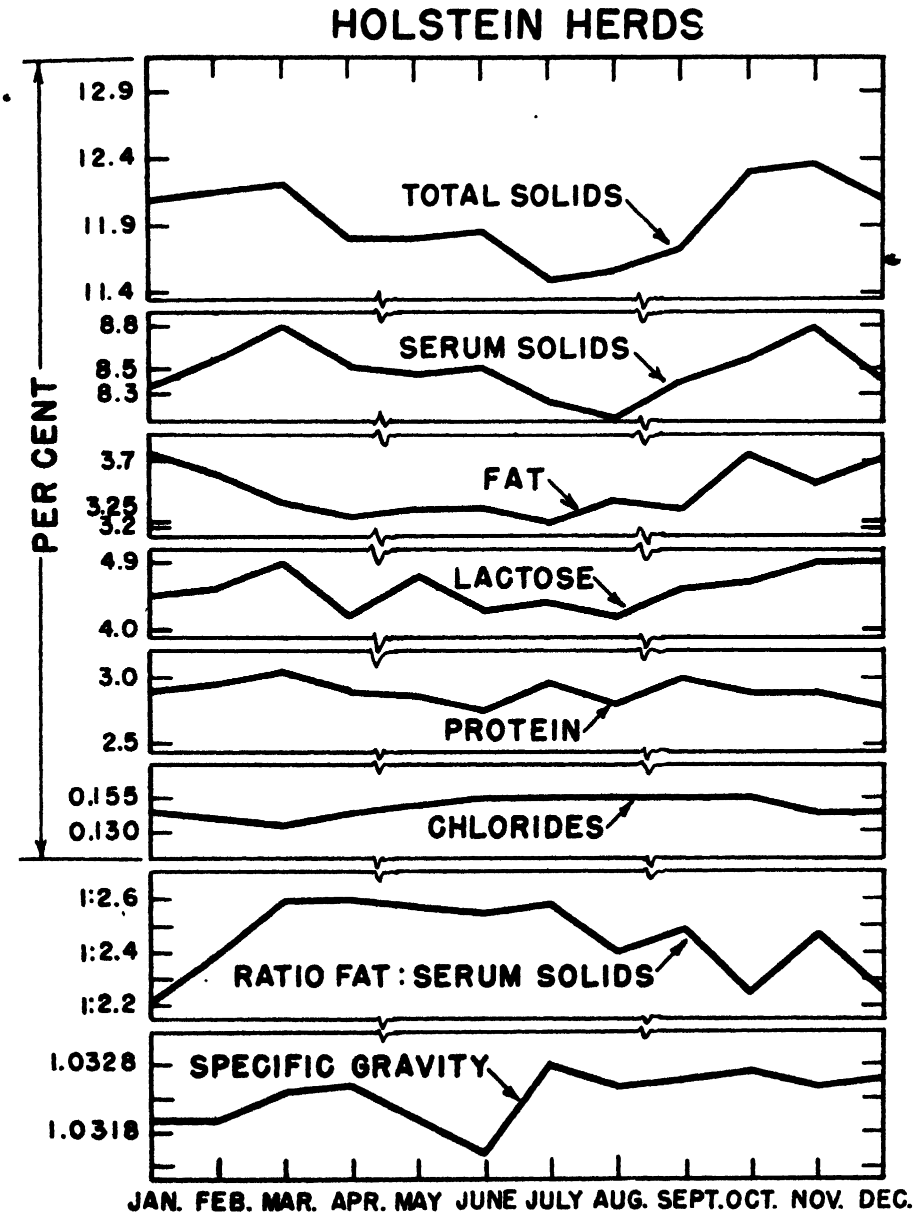

FIG. 3. Variations in constituents of milk from Holstein herds by calendar months.

The average chloride content of the milk from the four groups of herds was higher in summer than in winter. The winter chloride values for the Jersey, Guernsey, Holstein, and mixed herds were 0.10, 0.12, 0.14, and 0.12 per cent, respectively. The corresponding values for the summer months

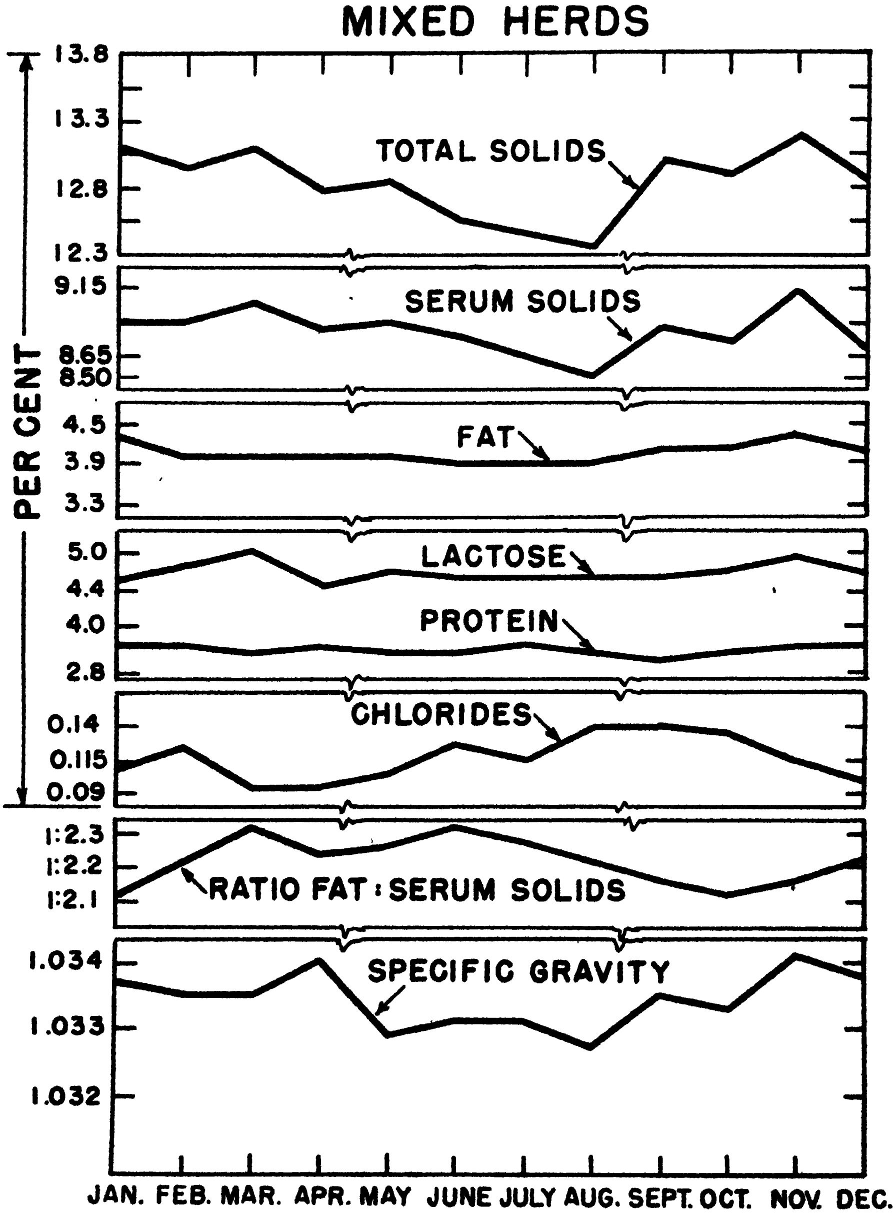

FIG. 4. Variations in constituents of milk from mixed herds by calendar months.

were 0.12, 0.13, 0.16, and 0.13 per cent. The values for other constituents of milk from the mixed herds also are intermediate between the low Holstein values and the high Guernsey and Jersey values. This is due to the greater numbers of Holstein cows in the mixed herds.

TABLE 2

Percentage of change of constituents of herd milk from winter to summer

Breed	Jersey	Guernsey	Holstein	Mixed
	(%)	(%)	(%)	(%)
Constituent				
Total solids .	− 4.35	− 5.96	− 3.88	− 3.54
Serum solids .	− 1.39	− 5.94	− 1.42	− 2.73
Fat	− 9.78	− 6.02	− 9.51	− 0.52
Lactose	− 2.06	− 4.52	− 9.30	− 1.69
Protein	− 2.80	− 8.65	− 4.22	− 3.72
Chlorides	+ 14.17	+ 7.69	+ 10.62	+ 15.75

Three of the mixed herds were kept under marginal conditions of feeding. One of the purposes of this project was to compare the composition of the milk of these herds with that of milk from herds in which the cows were maintained in good physical condition. The total solids, serum solids, fat, lactose, protein, and chloride values and specific gravity for the herds in good physical condition were 12.7, 8.8, 4.0, 4.7, 3.2, and 0.13 per cent, and 1.0327, respectively. The corresponding values for the marginal herds were 12.5, 8.8, 3.7, 4.6, 3.2, and 0.13 per cent, and 1.0333 specific gravity. These data show that milk from marginal herds was slightly lower in total solids, fat, and lactose. This would indicate that the cow's body must be in a very poor physical condition to produce milk significantly lower in each of the constituents.

DISCUSSION

The difference between maximum and minimum values of each constituent of herd milk shown in table 1 is less than corresponding values for milk from individual cows shown in the first part of this series (1). In the second paper of this series (2), it was noted that individual cows may produce milk abnormally low in serum solids although well above the minimum in fat. This is true of herd milk, but to a smaller degree. Of 199 samples collected from ten herds (two Jersey, one Guernsey, two Holstein, and five mixed) over a 2-year period, eleven samples were abnormally low in serum solids. The average fat and serum solids for the eleven samples were 4.42 and 8.08 per cent, respectively.

The results of this study are in agreement with the work of Gaines (3). The average ratio of fat to protein varied from 1:0.721 for milk from Jersey herds to 1:0.830 for Holstein herd milk. Milk from mixed herds had a ratio of 1:0.813.

SUMMARY

(1) Total solids, serum solids, fat, and protein values were highest in winter and lowest in summer.

(2) The milk from Jersey and Guernsey herds, containing 4.91 and 4.36 per cent fat, respectively, showed no definite trends in lactose content in re-

lation to other constituents. The milk from the Holstein and mixed herds had a slightly lower lactose value during the summer, with an increase in the fall and winter months.

(3) The serum solids content of milk from Holstein cows was below 8.5 per cent during 6 months of the year. This decline occurred in summer and was accompanied by decreases in total solids, fat, and lactose. Serum solids content of the milk from Jersey, Guernsey, and mixed herds also averaged lowest in summer, but was still above the legal minimum, except for mixed herds, which had an average of 8.48 per cent serum solids in August.

(4) The average chloride values were 0.152 for the Holstein herds, 0.134 for the mixed, 0.128 for the Guernsey, and 0.110 per cent for the Jersey herds. The chloride values were highest in summer and lowest in winter.

(5) Cows on marginal rations and consequently not in good physical condition produced milk which is considered normal in composition and averaged only slightly lower in total solids, fat, and lactose, essentially the same in serum solids, and slightly higher in the protein content and in specific gravity.

(6) Herd samples of milk representing one day's production may be abnormally low in serum solids but well above the minimum in fat.

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SOME EFFECTS OF FEEDING IODINATED CASEIN TO DAIRY COWS[1, 2]

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The recent availability of protamone (iodinated casein) for commercial use has placed further emphasis on studies dealing with the effects of this drug on lactating cows. Reports of research in Great Britain as well as in this country have already appeared in the literature (1, 3, 4, 5, 6, 7, 9, 10, 11, 12, 14, 15).

Early in 1946 studies were initiated at this Station with special emphasis on the composition of the milk and blood of animals before and after treatment with iodinated casein. Work reported by Archibald (1) included the determination of total solids, ash, fat, lactose, total protein, casein, and lactalbumin plus globulin in milk. The albumin-globulin fraction was obtained by subtracting the casein from the total protein content of the milk, but was not corrected for the non-protein nitrogen fraction. A decrease of from 1 to 33 per cent in casein occurred when cows were given 15 g. of iodinated casein daily for 4 and 5 weeks. The lactalbumin plus globulin fraction seemed to increase roughly in proportion to the decrease in casein. Only small changes were noted in the other components of the milk which were determined. A wide variation in milk yield response was reported.

While the present work was in progress a report by Van Landingham *et al.* (15) appeared which indicated little change in total, casein, and noncasein nitrogen in the milk from cows subjected to an iodinated casein regimen. However, increases occurred in the heart rates, respiration rates, milk and fat production, and per cent fat. An earlier report from the West Virginia Experiment Station (14) indicated that the milk vitamin C content decreased as much as 33 per cent when cows were fed iodinated casein.

The objective of the present study was to determine what alterations, if any, take place in the milk from cows receiving iodinated casein, with particular emphasis on the milk and blood nitrogen distribution. In addition, records were obtained on body weights, heart rates, and the extent and duration of response.

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METHODS

The work conveniently is divided into three trials involving four cows per trial. All animals used were purebred Jerseys or Holsteins from the University herd. Pertinent data regarding the individual animals are given in table 1. Individual milk samples (night-morning composites) were taken at the start and at 2- or 3-week intervals during the course of each experiment. Blood samples (approximately 100 ml.) were drawn into potassium oxalate from the jugular vein. These blood samples were taken at precisely the same time for each cow with reference to the milk sample, namely 6 hours after the morning feeding and on the same day that the morning sample of the night-morning milk composite was obtained.

TABLE 1

Status of cows at start of experiment and response in milk production

Cow no.	Age (years)	Stage of lactation (months)	Stage of gestation (months)	No. of lactations	Milk yield at start* (lbs./day)	Maximum response		Duration of response‡ (weeks)
						Lbs. milk per day†	Week of maximum	
Trial I—Feb. 14 to June 30, 1946								
J 729	3.0	4	1	1	13.5	+ 3.5	7	12
J 731	2.8	3	2	1	17.2	+ 4.3	2	7
H 228	5.0	9	Open	2	24.1	+ 9.0	3	
H 258	4.5	6	3	2	31.9	+ 10.7	3	11
Trial II—June 6 to Sept. 11, 1946								
H 240	4.7	14	Open	2	24.8	+ 3.3	2	7
H 255	5.5	10	Open	2	19.9	+ 2.9	7	8
H 330	3.1	8	1	1	27.6	+ 4.6	2	7
H 1587	8.3	10	Open	5	26.4	+ 12.1	2	9
Trial III—Nov. 21, 1946, to March 26, 1947								
H 552	7.0	8	Open	3	31.5	+ 11.3	3	15
H 1503	9.6	18	Open	5	16.7	+ 10.1	6	15 +
H 1723	7.8	11	Open	4	24.1	+ 8.7	3	6

J = Jersey, H = Holstein.

* Daily average for 7 days.

† Daily average for 7 days (during iodinated casein supplementation) minus milk yield at start.

‡ Week when average milk yield dropped below starting value.

Total solids were determined by drying 5-ml. aliquots of milk on cotton pads in a steam oven (98° C.) for 4 hours. The factor 5.15 (5 ml. × 1.03) was taken as the weight of milk before drying. Milk fat values were obtained by the Babcock method in trials I and II and the Mojonnier solvent extraction procedure (2) in trial III. Skim milk, casein, whole blood, and plasma nitrogen all were determined by the Kjeldahl procedure. Milk and blood non-protein nitrogen filtrates were prepared using tungstic acid, and the nitrogen content was obtained on aliquots of the filtrates by the Kjeldahl method. Milk and blood urea and ammonia nitrogen were determined on the tungstic acid filtrates, with and without urease incubation, after which the ammonia was distilled into standard acid after liberation with borax. Milk vitamin C was determined with the indophenol reagent (13). All milk samples were acidified and titrated with the dye within 30 minutes from the time of collection. Blood vitamin C was determined according to the method of Mindlin and Butler (8).

Heart rates were obtained by feeling the posterior tibial artery on the medial surface of the tibia at a point 8 to 10 inches above the hock joint. After the normal or starting values for the various milk and blood components were determined, the ration was supplemented with 15 g. of iodinated casein once each day by mixing it with the grain concentrate fed each morning. All cows received approximately 15 lbs. of corn silage twice daily, plus mixed hay *ad libitum,* in addition to the measured amount of grain concentrate (14 per cent protein) fed twice daily just prior to milking.

TRIAL I

Milk and blood analyses actually were made at intervals of 2, 4, 6, 8, 10, and 12 weeks after supplementation with iodinated casein was started. However, in order to conserve space, the results are tabulated for the start and at 6 and 12 weeks only (table 2). Cow no. 228 was lost to the experiment after 6 weeks, due to the development of an inflamed udder which was diagnosed as mastitis. In order to determine the effect of a higher intake of protein on the milk and blood nitrogen distribution, soybean meal was added to the grain concentrate so that the protein in the concentrate was increased from approximately 14 to 20 per cent during the 2-week period from the eighth to the tenth week of this trial. The only effect noted was an increase in the milk and blood nonprotein nitrogen values.

During the last 2 weeks of trial I, the dosage of iodinated casein was doubled in an attempt to delay the drop in production which was taking place after the initial high level attained during the first 6 weeks of treatment. In spite of the increased dosage of 30 g. per day per cow, the drop in production persisted, and at the end of the twelfth week the treatment was discontinued. A further sharp decrease in production followed (except for cow no. 729).

Tables 1 and 2 show that increases in milk production were obtained and the greatest responses to iodinated casein were evident in the two Holstein cows. The fact that the two Jerseys were younger cows may explain the relatively small response. It also is possible that the Jerseys were smaller animals with faster initial heart rates and hence would not be expected to

TABLE 2

Trial I. Effect of feeding iodinated casein on milk and blood nitrogen distribution, vitamin C content, and milk yield, fat, and solids-not-fat

Determination	Unit	Jersey no. 729			Jersey no. 731			Holstein no. 228			Holstein no. 258		
		At start	6 weeks	12 weeks	At start	6 weeks	12 weeks	At start	6 weeks	12 weeks	At start	6 weeks	12 weeks
Milk yield—increase (+) or decrease (–)	lbs./day	12.8	+ 3.7	+ 2.0	16.8	+ 2.2	– 5.1	25.8	+ 6.9		32.7	+ 10.0	– 3.4
	%		+ 29	+ 16		+ 13	– 30	.	+ 27		. .	+ 30	–10.0
Milk fat	lbs./day	0.65	0.73	0.77	0.94	1.08	0.70	0.96	1.31		1.05	1.49	1.29
	%	5.1	4.4	5.2	5.6	5.7	6.0	3.7	4.0		3.2	3.5	4.4
Milk solids-not-fat	%	9.48	9.43	9.09	9.72	9.78	9.35	8.33	8.87		8.15	8.94	8.82
Milk vitamin C	mg./l.	22.6	18.3	11.0	30.1	22.4	12.4	20.2	14.5		15.5	14.1	12.9
Blood vitamin C	mg./100 ml.	0.36	0.47	0.35	0.47	0.54	0.43	0.35	0.37		0.33	0.44	0.34
Skim milk nitrogen	g./100 ml.	0.65	0.60	0.55	0.69	0.64	0.63	0.57	0.57		0.51	0.50	0.51
Casein nitrogen	g./100 ml.	0.51	0.47	0.42	0.54	0.50	0.48	0.42	0.43		0.39	0.39	0.42
Milk albumin plus globulin nitrogen	g./100 ml.	0.10	0.10	0.09	0.12	0.11	0.12	0.12	0.11		0.09	0.09	0.06
Milk non-protein nitrogen	mg./100 ml.	37.0	29.2	25.3	30.0	32.7	26.2	32.4	27.8		33.5	21.9	27.4
Blood total nitrogen	g./100 ml.	2.67	2.80	2.54	2.69	2.77	2.50	2.75	2.71		2.91	2.71	2.79
Blood plasma nitrogen	g./100 ml.	1.14	1.09	1.12	1.11	1.09	1.04	1.27	1.16		1.25	1.14	1.15
Blood non-protein nitrogen	mg./100 ml.	28.4	21.1	15.6	25.1	24.3	17.4	24.5	21.1		30.2	23.0	19.2

experience as much stimulation in metabolic rate when fed iodinated casein. In this respect it might be predicted that a dual-purpose breed of cattle, such as the Milking-Shorthorn, would give a larger response in production when treated with the drug inasmuch as these animals have a lower metabolic rate than the dairy breeds. It also has been shown (4, 5) that as the time for parturition approaches, the response to iodinated casein diminishes. Any or all of these factors may have been involved.

In connection with the inhibitory effect due to pregnancy, the question of the duration of response by open cows is raised. The results of subsequent trials on lactating non-pregnant cows were variable. Five out of seven open cows reported in this paper decreased in milk yield after the usual period of response had taken place. Cow no. 1503 was open and had responded for a period of 15 weeks when feeding of the drug was discontinued.

Decreases of from 30 to 63 per cent in the milk vitamin C were obtained and these results confirm the work of Van Landingham. Blood vitamin C showed no tendency to decrease in a corresponding manner. Even the subcutaneous injection (into cows receiving iodinated casein) of ascorbic acid at a level of 50 mg. per 100 lbs. body weight daily for 7 days failed to raise the milk vitamin C to a normal level.

TRIAL II

On the basis of the results of the first trial it was decided to repeat the experiments with four Holstein cows in an attempt to get the greatest possible response in milk yield. Recognition of any changes in the various components of the milk and blood for which analyses were being made would be facilitated. This trial was conducted during June, July, and August, in contrast to the first trial which was run in the early spring while the cows were confined indoors. In trial II the cows were out on pasture during most of the run in addition to being fed hay *ad libitum*, grain or corn silage, and a measured quantity of grain supplement.

Work reported by Seath *et al.* (12) in which cows in Louisiana were fed iodinated casein during the hot summer period revealed that with or without iodinated casein, body temperatures were 104.6 to 105° F. when the atmospheric temperature ranged around 93.5–96.5° F. The question of the advisability of feeding compounds such as iodinated casein to Louisiana milk cows during the warmer months of the year was raised.

Descriptions of the four cows used in trial II are given in table 1. In this experiment milk and blood samples were obtained at the start and at 3- and 6-week intervals only. Results of the analyses are tabulated in table 3.

At times during this trial the environmental temperature reached 90° F. or higher, and all four cows visibly were suffering from what appeared to

TABLE 3

Trial II. Effect of feeding iodinated casein on milk and blood nitrogen distribution, and milk yield, fat, and solids-not-fat

Determination	Unit	Holstein no. 240			Holstein no. 255			Holstein no. 330			Holstein no. 1587		
		At start	3 weeks	6 weeks	At start	3 weeks	6 weeks	At start	3 weeks	6 weeks	At start	3 weeks	6 weeks
Milk yield—increase (+) or decrease (−)	lbs./day	25.5	+1.5	+0.1	19.6	+1.3	+1.1	29.2	+1.8	−0.6	24.0	+ 6.1	+10.5
	%	. .	+6	0		+7	+6		+6	−2		+25	+44
Milk fat	lbs./day	0.87	0.97	0.72	0.61	0.65	0.58	0.93	1.33	1.14	0.86	1.05	1.10
	%	3.4	3.6	2.8	3.1	3.1	2.8	3.2	4.3	4.0	3.6	3.5	3.2
Milk solids-not-fat	%	8.60	8.74	8.51	7.89	7.80	7.52	8.78	8.96	8.87	8.65	8.33	8.31
Skim milk nitrogen	g./100 ml.	0.54	0.49	0.50	0.48	0.45	0.43	0.48	0.51	0.48	0.54	0.47	0.49
Casein nitrogen	g./100 ml.	0.42	0.37	0.38	0.37	0.34	0.32	0.40	0.40	0.38	0.39	0.33	0.35
Milk albumin plus globulin nitrogen	g./100 ml.	0.09	0.09	0.09	0.08	0.08	0.08	0.05	0.08	0.07	0.12	0.11	0.11
Milk non-protein nitrogen	mg./100 ml.	29.6	27.4	30.9	27.6	25.4	27.1	33.8	25.2	25.2	30.2	24.7	27.7
Blood total nitrogen	g./100 ml.	2.89	2.80	2.70	2.71	2.47	2.69	2.65	2.46	2.78	2.58	2.34	2.55
Blood plasma nitrogen	g./100 ml.	1.13	1.09	1.09	1.23	1.16	1.16	1.14	1.04	1.16	1.23	1.21	1.28
Blood non-protein nitrogen	mg./100 ml.	10.1	14.7	20.9	14.4	15.2	18.2	19.7	17.4	17.7	17.4	15.2	18.3

be overheating, as indicated by failure to consume all of the ration (off feed), very fast respiratory rates (120 per minute), and fluctuations in the milk yield. The fact that all of the grain concentrate was not being consumed introduced a variable, since the exact intake of iodinated casein was difficult to determine.

An inspection of tables 1 and 3 shows that the increases in milk production were not as great (with the exception of cow no. 1587) as were obtained with the cows used in trial I.

TRIAL III

While trial II was in progress a publication by Blaxter (4) from England was released in which it was concluded that the greatest increases in milk production resulted from older cows, possibly correlated with a more sluggish or less active thyroid gland in older animals. Therefore, a third trial was inaugurated involving four Holstein cows all 7 years of age or older. Unfortunately one of the cows developed a rumen impaction just prior to being placed on the experiment, so only three cows were used. Table 1 includes a description of these animals as well as the extent and duration of response.

Trial III was conducted during the period November, 1946, to March, 1947. The animals were confined indoors the entire period and received the typical winter ration fed in trial I. The results of the milk and blood composition studies are tabulated in table 4. Blood hematocrit data were included in the blood analyses as a check upon the constancy of the blood volume. Also, milk and blood urea and ammonia nitrogen values were determined in this trial. While the ammonia nitrogen content of the milk and blood was quite constant, the urea values decreased by 20 to 40 per cent after 6 weeks on iodinated casein. The three cows in trial III were treated with the drug continuously for 15 weeks (tables 1 and 5). Two of the three cows (nos. 552 and 1503) showed an increased milk yield during the entire period which was equal to or greater than the starting value, whereas cow no. 1723 responded for 6 weeks only and then the yield gradually declined below the starting value. The actual weekly average milk yields at the end of the fifteenth week were 31.5, 19.7, and 16.1 lbs. of milk per day for cows nos. 552, 1503, 1723, respectively. At this point the feeding of iodinated casein was discontinued and after 2 weeks the production had dropped to 17.1, 10.4, and 7.1, respectively. These figures emphasize the precipitous drop in production which took place when administration of the drug was discontinued. These results suggest that once dairy cows are subjected to a drug such as iodinated casein, it should be fed continuously even after the period of response has passed and production is declining gradually. Perhaps the thyroxine being supplied via iodinated casein has caused the elaboration of thyroxine by the thyroid gland of the animal to cease; if the

TABLE 4

Trial III. Effect of feeding iodinated casein on milk and blood nitrogen distribution, and milk yield, fat, and solids-not-fat

Determination	Unit	Holstein no. 552			Holstein no. 1503			Holstein no. 1723		
		At start	3 weeks	6 weeks	At start	3 weeks	6 weeks	At start	3 weeks	6 weeks
Milk yield—increase (+) or decrease (−)	lbs./day	33.2	+ 11.7	+ 7.3	16.2	+ 9.2	+ 11.7	23.3	+ 7.5	+ 1.9
	%		+ 35	+ 22		+ 57	+ 72		+ 32	+ 8
Milk fat	lbs./day	1.31	1.79	1.61	0.51	0.79	0.90	0.78	1.00	0.94
	%	3.95	3.98	3.97	3.16	3.10	3.21	3.33	3.25	3.73
Milk solids-not-fat	%	9.05	9.54	9.07	7.79	8.16	8.29	7.99	8.26	7.87
Skim milk nitrogen	g./100 ml.	0.64	0.65	0.62	0.54	0.52	0.49	0.53	0.51	0.46
Casein nitrogen	g./100 ml.	0.50	0.52	0.48	0.44	0.39	0.36	0.38	0.37	0.34
Milk albumin plus globulin nitrogen	g./100 ml.	0.10	0.09	0.11	0.06	0.10	0.10	0.11	0.10	0.10
Milk non-protein nitrogen	mg./100 ml.	34.7	35.4	28.8	36.7	31.4	32.2	35.4	36.5	22.4
Milk urea nitrogen	mg./100 ml.	15.5	14.2	12.5	21.4	25.6	13.4	20.7	18.9	12.0
Milk ammonia nitrogen	mg./100 ml.	1.79	1.23	1.89	1.62	0.95	1.30	1.85	1.46	1.35
Blood total nitrogen	g./100 ml.	2.65	2.74	2.65	2.92	2.76	2.97	2.85	2.91	2.79
Blood plasma nitrogen	g./100 ml.	1.43	1.36	1.32	1.33	1.23	1.28	1.29	1.29	1.30
Blood non-protein nitrogen	mg./100 ml.	25.4	21.3	15.5	32.8	23.0	19.4	29.8	22.3	14.0
Blood urea nitrogen	mg./100 ml.	15.6	15.1	12.2	23.3	18.2	13.1	22.3	17.4	14.6
Blood ammonia nitrogen	mg./100 ml.	1.62	1.18	1.78	1.62	1.57	1.78	1.96	1.62	1.46
Blood hematocrits	% cells	28.1	31.3	30.3	37.0	33.0	37.5	35.0	39.0	34.5

TABLE 5

Milk yield after feeding of iodinated casein discontinued

Cow no.	Time in weeks		Calculated production†	Actual production‡
	Treated	Untreated*	lbs. milk/day	lbs. milk/day
J 729	12	2	10.1	10.1
J 731	12	2	12.8	6.3
H 258	12	2	23.8	16.1
H 240	7	7	19.8	12.0
H 255	9	5	14.9	11.7
H 330	9	5	20.6	17.2
H 1587	10	4	18.5	11.0
H 552	15	2	22.3	17.1
H 1503	15	2	11.9	10.4
H 1723	15	2	17.0	7.1

* After treatment discontinued.

† An arbitrary value of 8% was taken as the normal average decline in milk yield per cow per month. The sum of the weeks treated plus weeks untreated is the week for which the calculated production is expressed.

‡ The average for the 7-day period during the last week of no treatment.

oral supply of thyroxine is discontinued suddenly, the animal may be unable to start elaborating its own requirements.

Without exception the animals in all three trials lost weight, varying from 49 to 201 lbs. after 6 weeks on an iodinated casein regimen. Decrease in body weight varied from 3.3 to 11.8 per cent of the starting weight. Whether the loss of weight can be prevented or diminished by feeding extra nutrients appears doubtful in view of the fact that cows nos. 729, 255, and 330 lost weight even when the intake of grain supplement was 40, 46, and 30 per cent. respectively, in excess of the calculated requirements (table 6).

DISCUSSION

Every attempt has been made in this work to obtain the greatest possible response from the feeding of iodinated casein to the cows as judged by the increase in pounds of milk produced per day. The greatest response came from cow no. 1503, in which a 72 per cent increase in milk was obtained in the night-morning composite sample taken 6 weeks after the supplementation of the drug started (table 4). Based on the weekly average milk yield (table 1), this cow showed an increase of 60 per cent in milk production during the sixth week of the experiment. Poorest responses resulted in trial II, conducted during the summer months, in which three of the four cows responded to the extent of only 13, 15, and 17 per cent, whereas cow no. 1587 experienced an increase of 46 per cent in milk yield. Cow no. 1587 was over 8 years of age, which may explain a greater response as compared to cows nos. 240, 255, and 330, which were considerably younger. These results tend to confirm the work of Seath (12) showing that the use of iodinated casein is highly questionable during hot weather.

TABLE 6

Heart rates and body weights vs. food intake

Cow no.	Heart rates (beats/min.)				Body weights (lbs.)				Ration level	
	At start	3 weeks	6 weeks	Increase (6 wks.)	At start	3 weeks*	6 weeks*	% loss (6 wks.)	lbs. grain /day	% over or under feeding†
J 729	84	90	87	+ 3	1077	− 68	− 72	6.7	8	+40
J 731	82	90	89	+ 7	800	− 35	− 67	8.4	10	+34
H 228	67	80	86	+19	1700	−127	−201	11.8	10	0
H 258	79	87	89	+10	1454	− 37	− 64	4.4	12	−11
H 240	71	79	78	+ 7	1479	− 43	− 49	3.3	12	+29
H 255	73	81	85	+12	1721	− 74	−105	6.1	12	+46
H 330	81	88	96	+15	1464	−118	−150	10.2	14	+30
H 1587	67	82	85	+18	1586	−116	−130	8.2	14	+25
H 552	76	85	90	+14	1526	− 47	− 59	3.8	14	+12
H 1503	64	73	91	+27	1823	− 50	−108	6.0	8	+ 9
H 1723	64	78	75	+11	1860	− 69	− 68	3.6	14	+32

* Weight lost compared to starting weight.

† Calculated on the basis of 1 lb. grain concentrate per 3 lbs. of milk per cow per day, based on the average milk yield for the first 6 weeks on iodinated casein.

Little if any correlation between heart rates and the level of concentrate feeding was apparent (table 6), contrary to the results of Moore (9). As the age of the cow increases, a greater response in milk production apparently can be expected. This is in harmony with the findings of Blaxter (4).

The values for milk solids-not-fat were quite variable and showed no definite trend in any one direction. There was a definite increase in both the per cent and pounds of butterfat produced by nine of the eleven cows. The two possible exceptions were cows nos. 240 and 255 in trial II, conducted during the summer months.

If the values for skim milk nitrogen for each cow when the maximum increase in milk yield occurred are compared with the starting values, there was a decrease in eight cows, the largest decrease being 0.05 g. of nitrogen per 100 ml. or 0.32 per cent protein (0.05×6.37). Cow no. 228 showed no difference, and cows nos. 330 and 552 had respective increases of 0.03 and 0.01 g. of nitrogen per 100 ml. Making the same comparisons for casein nitrogen, the values decreased in seven cases, were unchanged in three cases, and increased in one instance. The average decrease was 0.04 g. nitrogen per 100 ml. milk or 0.25 per cent protein. The values for albumin plus globulin nitrogen were practically unchanged, but the milk non-protein nitrogen values decreased for eight of eleven cows.

No definite trends occurred in the blood total nitrogen but the blood plasma nitrogen values showed a possible slight downward trend. Blood urea values decreased to the same extent as the milk urea values (20 to 40 per cent). Little change was found in the blood ammonia, and the values were practically identical with those for milk ammonia nitrogen.

The results of the present study, as well as the data of other workers, indicate only slight alterations in the milk from cows fed iodinated casein, with the exception of some of the vitamins such as ascorbic acid, niacin, and riboflavin. The ascorbic acid and riboflavin values decreased considerably, whereas the niacin increased (7). Hibbs and Krauss (6) found a decrease in thiamin which increased above normal after cessation of feeding the drug. Perhaps the small changes in milk and blood constituents are in support of the belief that the primary effect of iodinated casein is to increase the metabolic rate and that an increase in milk yield is an indirect effect due to a greater flow of blood (and nutrients) through the mammary gland.

After a certain time (see table 1) the milk yield decreased regardless of whether or not iodinated casein was fed. This period of response varied from 6 weeks for cow no. 1723 to 15 weeks for cows nos. 552 and 1503. After the decrease starts and the feeding of the drug is discontinued, a very rapid drop in production ensues. Production usually levels off again considerably below the level of production calculated (8 per cent decrease per month) had no iodinated casein been fed (table 5). Whether a continuation of the drug supplementation (after a decrease in response sets in) would minimize the drop in milk yield requires more investigation. Reece (10) fed iodinated casein for periods up to 17 months, but the only statement pertaining to milk yield was that six out of nine cows produced more milk and three produced less milk during the period of feeding the drug than that secreted in a similar segment of either a previous or subsequent lactation period. Moore (9) fed iodinated casein for periods as long as 8 months and found that after cows had been treated 4 to 8 weeks there was a considerable loss in body weight and milk production declined sharply.

Several factors should be considered carefully by dairymen who are contemplating feeding iodinated casein to their cows. In the first place loss in body weight occurs, even when extra nutrients are fed. In many cases an appreciable drop in production (below the normal production calculated to correct for time) occurs after feeding of the drug has been discontinued. The duration of response (6 to 15 weeks) is relatively short considering the length of a normal lactation period. There also are seasonal limitations (hot weather appears to be deleterious), and limitations depending on the breed, age, and stage of lactation of the cows. Blaxter (4) concluded that the ideal use of iodinated casein was for a 2-month period in the early spring. Thus, after the period of response was ended, the cows would receive a stimulation from the intake of grass to counteract the impending decrease in production when the feeding of the drug was stopped. Finally, there is a marked individual variation in the degree of response which each cow treated undergoes.

All of the factors mentioned place considerable limitation on the use of iodinated casein. Still unknown and yet to be more fully investigated is the effect on health and normal productive span of feeding the drug to cows during successive lactations. The indiscriminate use of iodinated casein may lead to a negative rather than a positive over-all gain from a practical viewpoint. Under carefully controlled conditions, taking into account the status of each animal to be treated, it may yet be shown that increased milk production mediated by iodinated casein can profitably be realized. In the meantime considerable caution should be exercised in its use.

SUMMARY

Data have been secured on the effect of feeding 15 g. of iodinated casein daily to eleven dairy cows for varying periods of time and under various conditions. Increases in milk and butterfat production were obtained. All cows lost weight and had increased heart rates. Night-morning milk composites were obtained at 2- and 3-week intervals during the experimental period and analyzed for fat, total solids, skim milk nitrogen, casein nitrogen, non-protein nitrogen, urea and ammonia nitrogen, and vitamin C. Decreases of 30 to 63 per cent in vitamin C and of 20 to 40 per cent in urea

nitrogen and a maximum decrease of 0.05 per cent (0.32 per cent protein) in the skim milk nitrogen were found.

Blood samples were taken concurrently with the milk samples and analyzed for total nitrogen, plasma nitrogen, non-protein nitrogen, urea and ammonia nitrogen, vitamin C, and per cent cells. Blood urea nitrogen decreased.

The duration of response varied from 6 to 15 weeks, after which a marked drop in milk yield occurred after iodinated casein feeding was discontinued. Age and breed differences in response were noted. The limitations of iodinated casein for commercial usage are discussed.

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CHOLINE STUDIES WITH YOUNG DAIRY CALVES. I. RELATION OF THE CHOLINE CONTENT OF COLOSTRUM AND CALF BLOOD[1]

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The recognized importance of colostrum in the nutrition of young calves can be attributed, in part, to its high vitamin content. The high vitamin A content of colostrum is reflected in the vitamin A content of blood of the calf consuming it (6, 7). Colostrum also contains other vitamins in comparatively large amounts (11), but they have not received as much study as has vitamin A.

The indispensability of choline for some animals has been demonstrated, and its rôle in nutrition has been reviewed by Griffith (5) and by Best and Lucas (1). The value of choline in the nutrition of calves has been studied very little. Norton *et al.* (10) reported that additions of certain vitamins, including choline, to calves' diets produced no favorable results.

The occurrence of fatty livers has been the principal criterion of choline deficiency. The choline content of the blood has not been used extensively to indicate adequate dietary intake or normal choline metabolism. Luecke and Pearson (9) fed rather large amounts of choline chloride to sheep and dogs and found no accumulation in liver or plasma. Chaikoff *et al.* (2) have shown that the choline content of blood plasma is depressed in dogs which have been deprived of the external pancreatic secretions or which have been depancreatized and maintained with insulin.

Normal milk contains appreciable but not large amounts of choline (4, 8). It was thought that colostrum might contain considerably more choline than normal milk and affect the blood choline levels of newborn calves. This investigation was a study of the choline content of colostrum and of blood from calves for periods of 1 week following parturition.

EXPERIMENTAL

Method of choline analysis. The method of Engel (3) was modified and adapted to the analysis of liquid samples. Milk or blood in 10-ml. quantities was adsorbed on rolls of Reeve Angel no. 245 filter paper and dried in a Cenco vacuum oven at 80° C. The dried rolls were extracted 24 hours in a Soxhlet extractor with absolute methanol. The extract was evaporated to near dryness on a steam bath. The residue was treated with 25 ml. of an

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[2] Now at North Carolina State College of Agriculture, Raleigh.

aqueous solution of saturated barium hydroxide for 2 hours on a steam bath. The samples were cooled, neutralized to phenolphthalein with glacial acetic acid, and filtered by suction through an asbestos gooch into a large test tube (25 × 200 mm.). The flask and residue on the gooch were washed with small amounts of distilled water. The filtrate was treated with 6 ml. of 2 per cent Reinecke salt in methanol, and the contents of the tube were mixed and allowed to stand overnight in a refrigerator at 3–4° C. to allow precipitation of the choline reineckate. The choline reineckate was collected on a fritted glass gooch of medium porosity by filtering with suction. The tube and precipitate were washed three times with 2-ml. portions of cold (3° C.) absolute methanol. Suction was continued for a short time to remove the alcohol. The precipitate of choline reineckate adhering to the tube and on the gooch was dissolved in acetone and filtered into a volumetric flask. The acetone solution of choline reineckate was made to volume and the concentration was determined by means of a Beckman spectrophotometer using a wave length of 520 mμ.

It is recognized that Reinecke salt is not absolutely specific for precipitation of choline (1). The procedure was checked by adding known amounts of choline chloride to blood and milk which, upon analysis, gave satisfactory recoveries.

Choline chloride was used as a standard in the determinations, and all values are expressed in amounts of choline chloride.

Choline content of colostrum. The choline content of colostrum from five cows was determined. Care was taken that the calves did not nurse these cows. The first milking was made 4 to 5 hours following parturition and morning and evening thereafter. They were milked by hand for the first six milkings, and each milking was as complete as possible. After the sixth milking the cows were milked by machine. Immediately before the sixth milking 10 I.U. of oxytocin was injected into the jugular vein to aid

TABLE 1

Choline content of colostrum and milk
(mg. of choline chloride per ml.)

Sample	50B*	18P*	40B*	351†	618‡	Av.
1st milking . .	0.69	0.79	0.61	0.73	0.65	0.69
2nd milking ..	0.32	0.39	0.31	0.45		0.37
3rd milking	0.25	0.22	0.23	0.38	0.26	0.27
4th milking .	0.23	0.16	0.17	0.26	0.23	0.21
5th milking	0.16	0.17	0.14	0.23	0.21	0.18
6th milking .	0.14	0.19	0.12	0.21	0.21	0.17
1 week	0.10	0.17	0.13	0.13	0.18	0.14
2 weeks	0.11	0.15	0.13	0.11	0.18	0.14
4 weeks	. .. ...	0.12	0.10	0.16	0.15	0.13

* Holstein.
† Jersey.
‡ Guernsey.

TABLE 2

Calf blood choline levels from birth to 1 week of age
(mg. choline chloride per ml. whole blood)

Age	79B*	B584*	JP317†	B587*	P124†	B588*	B589*	Av.
Birth	0.16	0.22	0.19	0.14	0.22	0.22	0.11	0.18
1 day	0.21	0.22	0.20	0.18	0.24	0.22	0.16	0.20
2 days ..	0.23	0.24	0.26	0.20	0.24	0.21	0.19	0.22
7 days ...	0.26	0.22	0.31	0.21	0.26	0.23	0.22	0.24

* Holstein.
† Jersey.

in more complete removal of milk from the udder. It is doubtful if the injection at that time influenced the choline values obtained. The results are shown in table 1.

The average choline content of the colostrum of the first milking was 0.69 mg. per ml. By the sixth milking, the content had dropped to 0.17, and composite samples of morning and evening milk at 1 week averaged 0.14 mg. per ml., which is close to the value of 0.147 reported by Engel (4) and of 0.149 reported by Hodson (8) for normal milk.

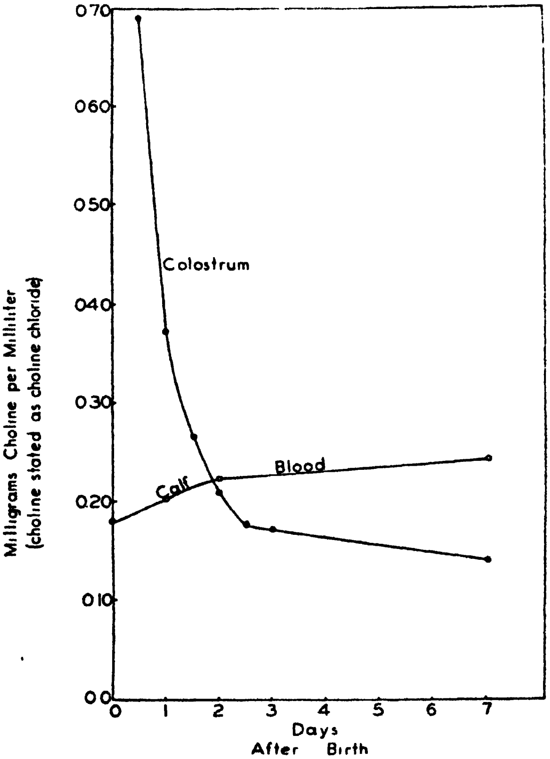

FIG. 1. The relation of the choline content of colostrum to the choline content of the blood of calves.

Choline content of calf blood. Preliminary data had indicated that the blood choline levels of calves were lower immediately following birth than several days later. In order to detect any relationship between the choline content of colostrum and calves' blood, blood samples were drawn from seven calves at birth, and at 1, 2, and 7 days of age. The calves either remained with their dams for 3 days following birth or were fed their dam's milk from a nipple pail for the same period. No attempt was made to compare the blood choline of an individual calf to the colostrum choline of its dam, the colostrum data in some instances being collected from cows other than dams of this group of calves.

The results are shown in table 2. The choline content of the calves' blood was found to increase throughout the week. The initial increase, while the colostrum choline content was high, was more rapid than later. Figure 1 gives a graphic comparison of the colostrum and calf blood choline levels.

DISCUSSION

It is logical to assume that the high vitamin content of colostrum aids in better nutrition of the newborn calf. The comparatively high choline content of the colostrum probably has a similar purpose. The fact that calves' blood increases in choline most rapidly following birth, while the colostrum choline content is high, is evidence concurring with this assumption.

The choline content of the initial colostrum is comparatively high, but the decline in amount of choline is quite rapid. The rate of decline is comparable to that of vitamin A (7) and is much more rapid than for thiamine and riboflavin (11).

SUMMARY

1. Immediately following parturition the choline content of colostrum was found to be comparatively high, averaging 0.69 mg. per ml. for the first milking. The choline content decreased rapidly with subsequent milkings, averaging 0.17 mg. per ml. at the sixth milking and 0.14 mg. per ml. at 1 week.

2. Calf blood choline levels were found to be low at birth, but increased throughout the first week. The increase in choline of the blood was most rapid while the colostrum was comparatively high in choline, indicating that the calf used the choline of the colostrum to increase the amount of choline in its blood.

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A NEW QUANTITATIVE METHOD FOR DETERMINING THE SOLUBILITY OF MILK POWDERS[1]

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Several methods have been proposed for determining the solubility of milk powders. Lampitt and Bushill (3) have divided the various methods into two general classes: (a) those in which an estimate is made of the amount of material which dissolves, and (b) those in which the amount of insoluble material is determined. Among the latter is the method of Lampitt and Hughes (4), which probably is the most nearly quantitative method that has been proposed. In this method, a weighed quantity of powder is shaken 3 minutes with water and the whole portion is transferred to a centrifuge tube. After centrifugation, the top layer is removed; then the supernatant fluid is decanted off and its total solids content determined. The sediment is weighed while moist, dried, and weighed again. The amount of moisture lost in drying and the figure for total solids in the supernatant liquid are used to calculate the amount of dissolved solids carried into the sediment by the moisture. This value then is subtracted from the dry weight of the residue in order to determine the true weight of undissolved matter.

The Standards Committee of the American Dry Milk Institute (5) considered the method of Lampitt and Hughes too cumbersome for practical use and proposed a method which, in modified form (1), is the one most commonly used at present for determining the solubility of dry milks. The present modified method will be referred to in this paper as the ADMI method. It consists of reconstituting a 10-g. sample of the powder with 100 ml. of water by means of vigorous mechanical agitation, centrifuging 50 ml. of the reconstituted sample in a conical graduated centrifuge tube, and reading directly from the graduations on the tube the volume of the washed sediment. Because the total volume of the powder plus water used in reconstitution is not considered, and also because the density of the sediment in the centrifuge tube will vary quite widely from powder to powder, the results obtained are not directly related quantitatively to the 10-g. sample of powder used in the test. This method apparently is satisfactory for routine use with non-fat dry milk solids, for which it was designed. For use with whole milk powder, much of which may be reconstituted to fluid milk before use, it lacks the sensitivity to distinguish reliably between a powder that is easily reconstituted and one that is difficult to reconstitute to satis-

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factory fluid form. This lack of sensitivity probably lies mainly in the vigor of the mechanical agitation employed to reconstitute the sample in the test. Howat *et al.* (2) have shown that variations in the speed and duration of mechanical agitation result in significant variations in the apparent solubility of the powder. In part they attribute the variations they found to peptization of denatured proteins. These investigators proposed a method based on the determination of the total solids in the supernatant liquid. They found, however, that for the more soluble samples, this method was less sensitive than the estimation of the volume of sediment.

In this laboratory the estimation of the amount of undissolved material also has been found more sensitive for differentiating between the more soluble powders than is the determination of the amount of dissolved solids. As the vigor of agitation in reconstitution is decreased, the sensitivity becomes greater. By washing the sediment once the necessity of determining the total solids on the supernatant liquid has been eliminated; thus the complexity of the method as compared to that of Lampitt and Hughes (4) has been decreased.

EXPERIMENTAL

In devising the solubility test the authors have tried to simulate as nearly as possible, in reconstituting the sample, the conditions of concentration, temperature, and agitation that probably would exist in the home kitchen if milk powder were to be reconstituted for beverage purposes. The detailed procedure that finally evolved, which we shall hereafter refer to as the "gravimetric sediment method", is as follows:

1. Weigh out a 5-g. sample of the powder to be tested and transfer it to a 125-ml. Erlenmeyer flask.

2. Add 35 ml. of distilled water tempered to 22° C. to the powder in the flask.

3. Immediately stopper the flask with a rubber stopper and shake for exactly 30 seconds. The shaking consists of four complete excursions per second, each excursion being an up and down motion through a distance of about 1 foot.

4. Immediately pour the reconstituted sample into a clean, dry, 50-ml. round-bottom centrifuge tube that previously has been weighed to the nearest milligram. (Steps 2, 3, and 4 must be completed before the next sample is reconstituted. Several samples, preferably not more than eight to twelve, may be accumulated at this point before the remaining steps are carried out.)

5. Centrifuge at the speed of a Babcock tester for 15 minutes (approximately 900 r.p.m. with a 15-inch centrifuge).

6. Decant into a graduated cylinder 30 ml. of the supernatant fluid. To retain the top film in the tube use a spatula or a rubber stopper notched to admit air at the top of the tube.

7. With 40 ml. of water at 22° C., rinse out the flask in which the sample was reconstituted, and add this water to the centrifuge tube.

8. With a wire, stir the water with the sediment for about 10 seconds.

9. Centrifuge again for 15 minutes at the same speed as before.

10. Using a spatula or the cut-away stopper described in step 6 to hold back any floating material, decant the wash water carefully until the first flocculent sediment appears at the pouring lip.

11. Dry the tubes containing the sediment overnight in an oven at about 95° C. and weigh the tubes and dried sediment. The tubes are dried in a reclining position about 5° from the horizontal.

12. The weight of the dry sediment is calculated as per cent of insoluble matter. This, then, subtracted from 100 per cent gives the solubility index in terms of per cent.

The effect of retention of the floating layer. During centrifugation of the reconstituted sample there develops a film or layer which, with some powders, attains a thickness of several millimeters. In previously described methods, this layer is considered as fat and is discarded. Inasmuch as the milk had been homogenized prior to drying, the quantity of this film frequently was too great to be accounted for by the separation of fat alone. In the samples showing the thicker top films, a considerable volume of air cells in the powder particles could be observed in microscopic preparations of the powder. It appeared reasonable that such air laden particles as were not soluble would retain the air during reconstitution and migrate to the top during centrifugation. On the other hand, if the film were due to migration of fat globules only, there would be a marked increase in the fat-to-protein ratio in the film as compared to the fat-to-protein ratio of the original powder.

TABLE 1

Relationship of fat globule size to the fat content of the top film

Powder no.	Av. volume of fat globules ($\times 10^{18}$)	Fat: protein ratio in:			
		Top film	Sediment	Middle milk	Dry powder
	(*cu. mm.*)				
25	1.70	8.07	0.74	0.87	0.99
40	1.98	6.96		0.91	1.01
48	0.32	3.34		0.81	0.91
38	0.25	3.06		1.14	1.19
52	0.22	2.70	1.12	0.93	1.01
15	0.26	1.46	1.24	0.89	0.99
8	0.24	1.28		0.77	
18	0.31	1.20	1.13	0.80	0.90

In order to determine the nature of the top film, the film was collected after the first centrifugation from tubes prepared in the normal manner for the test. The fat-to-protein ratio in this film was compared to that in the original powder and also to that in the unwashed sediment and in the middle portion of milk. The results are shown in table 1.

An attempt also was made to estimate the average size of the fat globules in the uncentrifuged milk and these data likewise appear in the table. The size of fat globules was estimated by the following method: 1 ml. of reconstituted milk (12.5 per cent solids) was diluted with 50 ml. of a warm solution of 5 per cent gelatin. A loop of this mixture was placed on a slide and covered with a cover slip. By means of a previously calibrated Whipple eyepiece, the size of all globules in a given field under the oil immersion objective of the microscope was estimated. The globules were grouped as to size and an average diameter was assumed. The groupings and the assumed diameters were: (a) greater than 2.5 microns (av., 5.0), (b) 1.0–2.5 (av., 1.75), and (c) less than 1 micron (av., 0.5). From the average diameter and the number of globules in each group, the average volume of all of the globules was calculated. At least twenty fields were examined for each sample.

TABLE 2

Effect of retention of the top film on the calculated solubility index

Sample no.	Solubility index		Lowering of index due to inclusion of top film
	Top film discarded	Top film retained	
1	92.2	89.9	2.3
4	85.4	83.9	1.5
7	98.3	93.9	4.4
9	93.0	83.6	9.4
11	96.4	93.5	2.9
12	98.8	97.6	1.2
15	95.6	93.3	2.3
16	92.3	90.4	1.9
17	86.7	84.9	1.8
18	79.8	76.7	3.1
20	80.9	79.2	1.7
21	89.6	89.6	0.0
22	98.6	85.5	13.1
28	99.1	96.9	2.2
38	98.8	96.9	1.8
40	98.4	96.2	2.2
41	98.8	97.0	1.8
42	99.5	96.0	3.5
43	98.2	92.9	5.3
44	99.4	96.6	2.8
48	99.0	96.5	2.5
50	99.7	98.0	1.7
52	98.6	95.3	3.3

Although the two samples with the larger fat globules showed considerable enrichment of fat in the top film, in most of the samples a large amount of protein had migrated to the top film. For that reason, the top film was regarded as insoluble matter and was retained in the test.

The effect of retaining the top film is shown in table 2, where the solubility indices with and without the top film are shown for 23 samples of powder. With powders no. 22 and no. 9, the amount of top film was excessively large. Microscopic examination revealed that both of these powders contained a large volume of air occluded in the powder particles. The retention of the top film lowered the solubility index by more than 1 per cent for all powders except no. 21, in which there was no measurable amount of top film. From table 2 it may appear that the retention of the top film places a drastic penalty on all powder samples. In spite of this penalty several samples were obtained that repeatedly showed a solubility index greater than 99.0 (see table 5). Only in the case of whole milk powder made from unhomogenized milk would the retention of the top film cause the results to be unduly low.

Effect of speed and duration of centrifugation. In the ADMI solubility test the samples are centrifuged at the speed of a Babcock centrifuge for 5 minutes. The authors found that after only 5 minutes at that speed neither the sediment nor the top film was sufficiently compact and tenacious to permit easy separation of the liquid without loss of insoluble matter. In order to determine the effect of the speed and duration of centrifugation, several trials were made with speeds of 900 and 2600 r.p.m. for 5 and 15 minutes. A few determinations were made with 10-minute and 30-minute periods of centrifugation. The results are shown in table 3.

TABLE 3

Influence of speed and duration of centrifugation on the solubility index

Sample no.	Centrifugation at 900 r.p.m.				Centrifugation at 2600 r.p.m.			
	5 min.	10 min.	15 min.	30 min.	5 min.	10 min.	15 min.	30 min.
4	88.0		85.9		82.4		82.0	
15	96.9		96.4	94.6	95.5		91.9	89.4
17	86.3	85.6	84.5		84.5	82.9	80.6	
18	81.9		79.4	77.9	77.6		78.9	78.2
37	99.0		97.9	96.8	96.6		91.0	87.8
43	94.3		92.3	89.5	90.3		86.3	84.4
50	99.3	98.7	98.2		97.2	93.2	91.8	

By increasing the time from 5 minutes to 15 minutes at a speed of 900 r.p.m., the solubility index of the different powders was lowered 2.1 to 0.5 points. Increasing the speed to 2600 r.p.m. caused a drop which, in the case of some powders, was quite marked. After 5 minutes at this speed the index was from 5.6 to 1.4 points lower than for the same period of time at 900 r.p.m. and from 3.5 to 0.0 points lower than for a 15-minute period at 900 r.p.m. Increasing the time to 15 minutes at the high speed caused a further marked decrease in index.

A few analyses of the top film obtained at speeds greater than 900 r.p.m. revealed considerably more migration of fat to the top film at the higher speeds. In order to keep fat migration to a minimum and yet get the top film and sediment sufficiently compact to facilitate easy separation of the liquid, a period of 15 minutes at 900 r.p.m. was adopted.

Influence of time in the wet phase. After the reconstitution of the sample the undissolved matter is in contact with the water and subject to loss by solution or dispersion until the wash water is finally decanted. In addition to the two 15-minute periods that the samples are in the centrifuge, considerable time also is required to carry out the various manipulations of the test. With a set of eight samples the time that elapses from the reconstitution of the first sample to the completion of washing of the last sample is approximately an hour. Sets of more than eight samples obviously would require more time.

To determine what effect such an increase in time would have upon the results, a set of eight replicate samples was weighed out. Duplicates from this set were reconstituted at intervals of 10 minutes and held in the centrifuge tubes until the last pair was started. Then all of the samples were centrifuged and the test completed on the entire set in the usual manner. By this procedure the first pair of samples was in contact with water 30 minutes longer than was the last pair. The results for four different powders are shown in table 4.

TABLE 4

Influence on the solubility index of time required to perform the test

Time elapsed from reconstitution to pouring off wash water	Powder no. 15		Powder no. 17		Powder no. 18		Powder no. 43	
	Solubility index	Increase due to time	Solubility index	Increase due to time	Solubility index	Increase due to time	Solubility index	Increase due to time
(min.)								
60	95.8	0.0	87.9	0.0	79.6	0.0	93.1	0.0
70	96.2	0.4	88.2	0.3	79.8	0.2	93.6	0.5
80	96.6	0.8	88.8	0.9	80.2	0.6	93.7	0.6
90	96.8	1.0	89.1	1.2	82.0	1.4	93.6	0.5

With powder nos. 15, 17, and 18 there was a steady rise in the solubility index as the period of time increased, indicating that more and more of the insoluble matter was dispersed as the time of contact increased. In view of these data it is suggested that for uniform results no set of samples be so large that the manipulation cannot be completed within about 1 hour after reconstitution.

Effect of temperature. A few trials were made to determine the effect of the temperature of the water of reconstitution upon the results of the test. Within the range of 19 to 30° C. the results were affected less by the initial temperature of the water than by the temperature of the room and the centrifuge in which the work was being conducted. The temperature of the liquid in the tubes very quickly approached that of the environment. Although no well-controlled data are available to support the assumption that environmental temperatures affect the test, observations indicated that the temperature should be maintained as closely as possible in the range of 20–25° C.

Determination of blank. Obviously the gravimetric sediment method always will give a solubility index less than 100. To determine the extent of the residue remaining in the tube even after centrifuging fresh homogenized milk, residue tests were made on twelve batches of milk taken after concentration in the vacuum pan and homogenization. In table 5 these data are compared with the solubility indices obtained on the corresponding fresh powders. The solubility indices obtained with the fluid milk samples ranged from 98.3 to 99.5. Generally, the less concentrated samples gave lower values than did the more concentrated samples. More efficient homogenization of the more viscous fluids may have resulted in a smaller amount of floating film in the test. In three instances the milk samples gave slightly lower solubility indices than did the corresponding powder samples, but the differences all are within the experimental error of the method.

TABLE 5

Comparison of solubility index of concentrated homogenized milk and the powder made from it

Batch no.	Total solids of conc. milk (%)	Solubility index	
		Milk*	Powder
96	21.40	99.1	98.1
97	39.90	99.2	99.1
98	22.84	99.0	98.7
99	38.90	99.4	98.9
100	23.36	98.3	97.5
101	42.00	99.1	99.2
102	39.02	99.1	99.4
103	26.45	99.2	99.3
104	22.70	99.1	98.5
105	39.35	99.4	99.4
106	23.26	98.6	97.9
107	40.09	99.5	99.3

* Concentrated and homogenized.

Accuracy of the test.[2] In table 6 are shown the variations that occurred when the same operator repeated the test several times on the same sample. The data were accumulated by three different operators. In the case of powder nos. 48, 50, and 52, both operator A and operator B made several tests. The greatest variation found was with powder no. 57, which in twelve determinations had a mean solubility index of 94.41 with a range of 3.42

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[2] Part of the data used in this analysis was kindly furnished by Mr. Louis J. Manus and Mr. V. H. Menzies, graduate students in this department who employed the test in their research work.

TABLE 6

Reproducibility of solubility indices

Powder no.	Operator	No. of trials	Mean solubility index	Difference between extremes	Standard deviation	Difference in means between A and B
15	A	5	92.86	1.90	0.35	-
18		5	76.93	1.64	0.76	-
25		4	92.96	0.50	0.22	-
38		4	96.79	0.32	0.14	-
40		5	95.92	0.66	0.28	-
48		6	96.38	1.36	0.49	-
50		7	97.73	1.26	0.49	-
52		6	95.33	1.58	0.68	-
46	B	12	96.95	3.16	0.96	-
48		11	96.59	2.10	0.63	+ 0.21
50		12	97.23	2.22	0.67	- 0.50
52		13	94.74	2.84	1.18	- 0.59
57		12	94.41	3.42	1.10	-
61		12	89.19	2.32	0.97	-
62		8	96.22	1.67	0.53	-
67	C	8	97.38	1.76	0.56	-
68		10	96.22	0.56	0.22	-
73		10	99.34	0.30	0.09	-
105		6	99.41	0.24	0.09	-
111		8	99.40	0.40	0.15	-

points between the extremes of 92.30 and 95.72. The standard deviation was calculated separately for each group of trials. While no analysis of variance was attempted, it can be seen by inspection of the standard deviations that two-thirds of the time the deviation from the mean can be expected to be less than ± 1. For powders in the range of greater solubility, such as nos. 73, 105, and 111, the expected deviation from the mean would be only 0.1 or 0.2 point.

TABLE 7

Comparison of the gravimetric sediment method with the ADMI method for determining solubility

Powder no.	Solubility index	
	Gravimetric sediment method	ADMI method
28	96.9	0.04
7	93.9	0.1
11	93.5	0.1
15	93.3	0.04–0.29
1	89.9	0.06–0.1
21	89.6	0.1
17	84.9	0.1–0.15
4	83.9	0.1
9	83.6	0.35
18	76.7	0.4

In the case of the three powders that were examined by both operator A and operator B, the difference between the means obtained by the respective operators was considerably less with every powder than was the difference between extremes found by each operator.

These results all were obtained by different men working in the same laboratory. It would be interesting to see how the results from different laboratories would compare.

Comparison with the ADMI method. For ten of the lots of whole milk powder on which the solubility index has been determined by the gravimetric sediment method, data on the ADMI solubility method also are available. The data obtained on these powders by the two methods are shown in table 7. Where two values are shown for the ADMI method, they represent results obtained on the same lot of powder by two different laboratories.

A high value obtained with the ADMI method should compare with a low value obtained with the gravimetric sediment method. For the last two samples listed in table 7, this relationship holds. For the other eight samples there is no significant difference found with the ADMI method; with the gravimetric sediment method, on the other hand, there are wide differences ranging from a high of 96.9 to a low of 83.9, a range of 13.0 points. According to the analysis in table 5, that difference is highly significant.

DISCUSSION

The gravimetric sediment method was designed primarily for use with whole milk powders, especially for those powders that may be sufficiently soluble for use as a beverage after reconstitution. In our experience, powders which give a solubility index of 97 or above are easy to reconstitute without mechanical stirring and give a product free of scum or graininess on the walls of the vessel and also free of a chalky feel in the mouth. In physical appearance the reconstituted milk from these samples cannot be distinguished from fresh homogenized milk. On the other hand, those powders with a solubility index of 90 or less always show some physical abnormality of one kind or another when reconstituted without vigorous mechanical agitation. As shown in table 7, the ADMI method fails to distinguish reliably between powders which, by the gravimetric sediment method, show a solubility index of 85 or greater. All of the powders listed in table 7, when tested by the ADMI method, would meet the requirements of premium grade powder with respect to solubility (1). Most of those powders, however, yield reconstituted milks that leave a grainy, chalky, or churned appearance on the walls of the glassware in which they are handled.

Experience with the gravimetric sediment method probably will dictate that certain changes be made in the procedure outlined in this paper. For example, the shaking procedure in the reconstitution of the sample is difficult to standardize. A standard mechanical shaking device might help to obtain more uniform results. Undoubtedly the temperature of the room in which the test is conducted influences the results significantly. A carefully controlled temperature would improve the accuracy of the method.

Even with its present imperfections, the test is the most sensitive of any that has come to the attention of the authors. Unfortunately, it penalizes whole milk powder that is made from milk that has not been homogenized. Since most of the whole milk powder manufactured in the United States is made from homogenized milk, however, this is not a serious defect. The gravimetric sediment method is offered here as a tool for use in research in the improvement of the solubility of milk powder.

SUMMARY AND CONCLUSIONS

A new method has been devised for determining the solubility of milk powders. It is sensitive to differences in solubility of powders which are too soluble to be differentiated by the method of the American Dry Milk Institute.

The new method differs from the ADMI method in the following features:

1. All of the reconstituted sample is used in the test instead of only a portion.
2. Reconstitution is by a standardized shaking procedure which puts into suspension less of the difficultly soluble material than does the vigorous mechanical stirring in the ADMI method.
3. The undissolved material separated by centrifugation of the reconstituted sample is dried and the amount is determined gravimetrically.
4. The results can be calculated to give the solubility index in terms of per cent of soluble material.

The method gives results that can be reproduced within a narrow range on repeated trials by the same operator or by different operators.

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THE NATURE OF SOME AIR-BORNE MATERIAL AROUND DAIRY ESTABLISHMENTS[1, 2]

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Efforts of dairy plant operators to prevent contamination of their raw and finished products with extraneous matter have been only partially successful. After continued operation of control programs, varying amounts of foreign matter still are found occasionally in the products. Presumably some sources of such material have not been controlled adequately. With the increasing tendency to study extraneous matter microscopically and emphasize its nature, small amounts of material become more significant.

The air around establishments where dairy products are produced and processed might be an important source of contaminating material; however, this potential source has received little attention. Although air frequently is examined to determine the prevalence of microorganisms, pollen, and dust particles and fumes of industrial origin, no reports have been seen on the incidence of other particles which sometimes are found in food products. The study reported herein was conducted to determine the general nature of air-borne material around dairy establishments. Such information would be helpful in planning control measures and in the interpretation of sediment test data on dairy products.

METHODS

Two methods of examination for air-borne material were used: (1) filtration, to detect particles floating in the air; (2) sedimentation, to give information on the type of material actually settling out of the air and for use when there were no facilities for operating the filtration procedure. While the two methods often were used concurrently, conditions did not always permit this arrangement. In general it was not intended to study particles smaller than could be readily observed with the 85× magnification of the wide field microscope. Although such smaller material undoubtedly is important from some standpoints, it is not of immediate concern as extraneous matter in dairy products.

Filtration method. In adopting a procedure for the detection of floating material, various described methods for the examination of air for bacteria, pollen, and industrial dust particles (1, 2, 3, 5, 6, 10) appeared to involve too small a volume of air or otherwise were impractical for the proposed study. After trying a variety of methods using suction fan equip-

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ment, a modification of the filtration apparatus of Silverman and Williams (11) using a tank-type vacuum cleaner finally was adopted. The dust bag and filter pad were removed from the tank to permit drawing a greater volume of air. A simple adapter, attached to the intake end of the cleaner hose by a piece of gooch rubber tubing, held the lintine disc in position and permitted filtration at the intake (fig. 1). The adapter consisted of a 1½-inch metal cylinder 1⅝ inches in diameter with a wire screen soldered in place to support a standard 1¼-inch lintine milk sediment test disc. A cylindrical clamp was provided to hold the disc in place, and paper gaskets were used to make a tight seal. Filtration at the intake, rather than within the tank or hose, avoided the possibility of contamination by residual material carried over from one run to another.

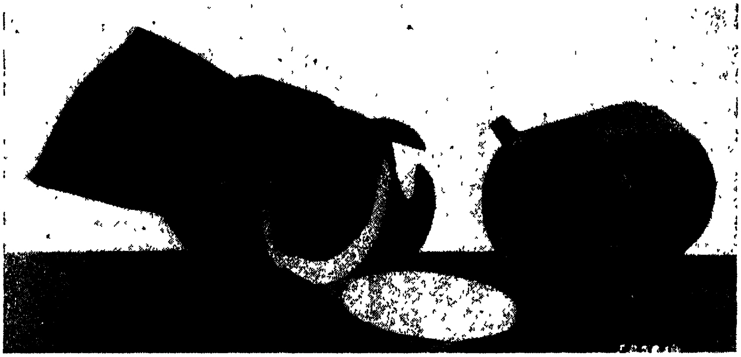

FIG. 1. Adapter of type used to hold lintine sediment test disc in place at intake end of vacuum cleaner hose.

With the lintine filter disc in place the apparatus was capable of drawing an average of 26.1 cu. ft. of air per minute.[3] Some variation in this rate was observed but it was small and not considered of importance in the study. The chief disadvantage of the vacuum cleaner apparatus was the dependence on electricity. Among its advantages were its portability and capacity to handle a relatively large volume of air. Although the lintine filter disc undoubtedly permitted some fine particles to be drawn through and others to become imbedded in it, this also is true of its use in sediment tests made on dairy products. In general, the soft face of the disc was effective in catching and holding air-borne particles until they could be examined microscopically, although some of the larger particles tended to drop off during handling unless considerable care was exercised. Efforts to improve the disc by adding some adhesive substance or by using different filtering material were not considered satisfactory. Furthermore, the possibilities of contaminating the disc were increased by such preparation and additional handling. The lintine discs, as purchased in packages, are ideal for air examination in the field. They are clean, handy, ready to use, and widely employed for checking extraneous matter in dairy products. In air studies they can be packaged, examined, and recorded in the same manner as when used with other products.

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[3] The apparatus was calibrated by members of the Kansas State College Department of Physics.

In filtering air with the tank vacuum cleaner apparatus, the adapter first was wiped thoroughly with a fresh clean lintine disc to remove any adhering particles. A new disc then was inserted and clamped in place. The intake, supported by a ring stand, was held in a generally vertical position about 18 inches above the floor or ground during a filtering period of 15 minutes. This period was selected after a few preliminary trials as being sufficient to collect adequate material for study under most conditions, and short enough to permit a number of tests to be made without excessively prolonging the operations. Limiting the trial to 15 minutes had the additional advantage that the motor did not overheat as it did during longer runs with the restricted air intake. The volume of air filtered at each operation was, accordingly, 391.5 cu. ft., with small variations. Precautions were taken that the intake was not adjacent to walls, ledges, floors, or other such sources from which it might pick up material. With the relatively long hose attachment it was possible to have the intake about 8 feet from the exhaust. Also in small spaces the exhaust air was directed into an open doorway. These practices minimized the drawing in of material stirred up by the exhaust air. At the end of the operation period the disc was removed carefully and tightly stapled in one of the common sediment test record cards with cellophane window. First, however, the cellophane was wiped carefully with a new, unused lintine disc to remove any extraneous particles. Such data as place of examination, weather conditions, and date were noted on the sediment card. The cards with discs were returned to the laboratory for microscopic examination and recording.

Another apparatus somewhat similar in action to the tank-type vacuum cleaner, and which did not depend on electricity, was a cleaner hose attached to the suction windshield wiper line of an automobile. The same adapter for holding the lintine filter disc was used. This equipment drew about 6 cu. ft. of air per minute with the motor idling at a fast speed. In preliminary trials this apparatus collected considerably less material than the vacuum-cleaner machine, even though used over much longer periods. Accordingly, this automobile-powered equipment was not used further during the investigation but may have some application under certain conditions.

Sedimentation method. Large petri plates (bottom portion 140 mm. x 20 mm.) were selected as the most satisfactory containers in which to collect particles settling from the air. These plates have the advantage of being easily protected before and after exposure, as well as being convenient for field work. No water or adhesive substance was used in the plates during exposure. Although some of the material collected might have drifted out of the plates later, the use of liquid would introduce other problems. The uncovered plates were placed on equipment or other surfaces and were left exposed for 2 hours during the day. This period is about the length of time that cream often is exposed in cans in cream stations during filling or before being covered by the operator. By using the 2-hour period, more tests could be made the same day and the exposed plates generally could be kept under observation. After exposure the plates were covered, labeled, packed, and returned to the laboratory. The material collected in the plates was removed with about 25 ml. of sediment-free water, using a wash bottle and a clean rubber policeman. The water from the plates was filtered through 1¼-inch lintine discs, using a simple filtering aid connected with a suction pump. The discs were mounted, dried, and then examined and filed in the same manner as those from the filtration method. Plates occasionally were examined microscopically after the removal of the collected material to see that no particles remained.

Examination of extraneous matter. The lintine discs were examined with a wide field microscope having magnifications of 21.2 ×, 42.5 ×, and 85 ×. Some of the more significant material found on the discs was identified through comparison with standards prepared from insect parts, rodent hairs, other hairs, vegetable substances, and other types of extraneous matter to which dairy products might be exposed at some stage of production, transportation, or manufacture. Familiarity with the microscopic appearance of these materials assisted in the identification of extraneous particles.[4] Identification of rodent hairs also was further facilitated by descriptive material (4, 7, 8). The extraneous matter on the lintine discs was grouped into nine general types, as indicated in table 1. This classification and the method of examination are generally the same as used by Miersch and Price (9) in a study on extraneous matter in cheese. The discs were checked as positive for each type of material encountered, irrespective of the number of particles present, except that rodent hairs were counted.

RESULTS

One hundred forty-four examinations for air-borne extraneous matter were made in various locations inside and outside four creameries, six cream stations, and seven cream producing farms. The farms were in the vicinity of Manhattan; the creameries and stations were located in a larger area, approximately 100 miles across. Both filtration and sedimentation methods of examination were used, but not necessarily concurrently. Where electricity was not available, only the sedimentation method was employed.

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[4] Assistance in part of this work was obtained from staff members of the College Departments of Entomology and Zoology.

TABLE 1

Nature of some air-borne material around dairy establishments

Type of material	Incidence of positive samples from each class of establishment					
	Creameries		Cream stations		Farms	
	Series 1* 30 filtration samples	Series 2 41 sedimentation samples	Series 3 15 filtration samples	Series 4 8 sedimentation samples	Series 5 23 filtration samples	Series 6 27 sedimentation samples
	% positive	*% positive*	*% positive*	*% positive*	*% positive*	*% positive*
Feather parts	16.7	7.3	13.3	25.0	8.7	7.4
Rodent hairs	13.3	9.8	26.7	12.5	17.4	14.8
Other hairs	23.3	17.1	20.0	37.5	34.8	44.5
Insect parts	56.5	26.8	46.7	12.5	34.8	18.5
Vegetable matter	36.7	43.9	66.7	50.0	78.3	92.6
Cloth fibers	56.7	73.2	73.3	62.5	47.8	63.0
Sand and clay	73.3	75.6	86.7	75.0	82.6	74.1
Coal dust	76.7	65.9	86.7	62.5	52.2	66.7
Unidentified	83.3	73.2	93.3	75.0	65.3	74.1

* Each series of examinations not necessarily conducted concurrently.

Examinations were made principally in late summer and fall. Climatic conditions were generally normal for the period, although there was considerable rain in early fall. Examinations were made under average weather conditions with extreme situations being avoided. Outdoor tests were not made during or immediately following rain.

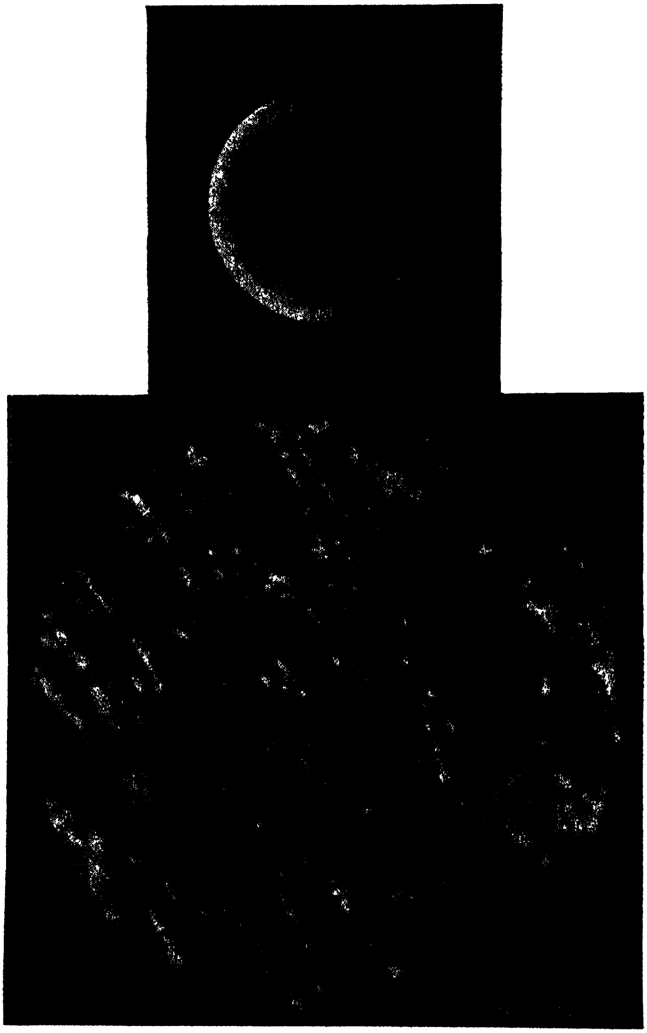

FIG. 2. *Upper*—Lintine disc after filtering approximately 390 cu. ft. air. Shows darkened disc but no large particles. *Lower*—Photomicrograph of portion of same disc showing rodent hair with upper portion out of focus. 50 ×.

Results are shown in table 1. The types of material indicated were present in the air around creameries, cream stations, and farms, and were found by both methods of examination. The table shows the percentage of samples positive for each type of material as obtained in each series. Most of the particles encountered were small and seldom could be identified without the aid of a microscope. This was particularly true in the case of the filtration examinations, where the lintine discs often became decidedly dark from the accumulation of small particles (fig. 2). The larger particles that were encountered usually were vegetable matter and most often were obtained by sedimentation in windy weather.

Coal dust, sand and clay, and cloth fibers generally were the most frequently encountered identified material. Vegetable matter was common, and hairs other than rodent type and insect parts frequently were found. Although rodent hairs and feather parts generally were less common than the other types of material, they were obtained around each class of establishment by both methods. Since longer testing periods would tend to increase up to 100 per cent the incidence of positive samples for each type of material, relative percentages should not be emphasized too greatly.

Weather conditions and season were observed to influence the amount and type of air-borne material. For this reason, and since all examinations were not made at the same time or under identical conditions, comparisons between results at creameries, cream stations, and farms cannot well be made. Considerably more extensive studies would be desired to permit these comparisons. Also, the two methods used hardly are comparable, since they differ in principle and time periods involved. Furthermore, they not always were used concurrently. For these reasons sedimentation data should not be compared with filtration data other than in a general manner to point out that the same types of air-borne material were demonstrated by each method.

DISCUSSION

The investigation showed that the same types of extraneous matter sometimes found in sediment tests of dairy products are present in the air around creameries, cream stations, and farms. The materials collected in exposed petri plates would indicate that some might settle onto exposed equipment and into milk or cream during the various stages of production and marketing.

Observations made during the investigation indicated that the amount and type of air-borne material varied with weather and seasonal conditions, as might be expected. After rain had washed the air and before the soil became dry on the surface, relatively little material was obtained, particularly outdoors. Also, with the onset of colder weather in the fall, the incidence of insect parts decreased. More material was collected in dry windy weather, particularly outdoors, since much of it not normally air-borne was then carried about.

Of the various types of extraneous matter encountered in air, the insect parts and rodent hairs presumably are of most significance and were found more frequently than was anticipated. Since the frequency with which rodent hairs are reported in dairy products is seemingly larger than can be accounted for by the possibility of more direct contamination, air may be a source of contamination by such material. In creameries, rodent hairs were found most frequently in the dry storage room. None were detected in churn rooms or print rooms; one positive sample was obtained from a receiving room. Rodent hairs were not found outside the plants.

Cream stations present a more difficult problem in the prevention of contamination by rodent hairs and insect parts because of the relative location and size of the establishments. Too often cream stations are located not far from grain mills, feed storage rooms, or produce houses which may be infested with rodents and insects. Therefore, the best of housekeeping in the small room used as a cream station may be ineffective in controlling air-borne foreign material. For example, in one station studied, where the general state of cleanliness was about average, air examinations showed the presence of rodent hairs both in and outside the station. The material collected from the air also contained an unusually large number of particles later identified as corn cob fragments from a feed mill about 200 yards distant. These findings suggested an association between the feed mill, the corn cob fragments, and the rodent hairs, which is natural in view of the general infestation of farm corn cribs with rats and mice. Such corn probably would increase the rodent hair content of the air in the vicinity of the mill, particularly when material as large as cob particles is being blown about during processing. Later examination of the air near the mill by both sedimentation and filtration methods showed the presence of rodent hair fragments, even though no cob particles were observed in the air at the time. A sample of soil from the vicinity also showed similar fragments. Thus it is apparent that under such conditions the presence of rodent hairs in the air of a near-by cream station is practically beyond the control of the station operator.

Farms present a rather different situation. Rodents and rodent hairs are extremely common around corn cribs and feed storage. Their numbers around barns may be limited by the presence of cats. However, since the hairs of both rodents and cats are very similar, they may be confused with one another in material obtained from barn air. In distinguishing them one must rely on general familiarity with the two types of hairs, plus evidence of cats or rodents in the near vicinity. Thus it is possible that, in the data reported herein as pertaining to examinations of air on farms, some cat hairs may have been included as rodent hairs.

The information obtained suggests that greater attention should be given to the protection of dairy products and equipment used in all stages of production and marketing to reduce possible contamination from air-borne extraneous matter. The actual methods required would consist, mainly, of a more assiduous application of such practices as the complete covering and protection of milk, cream, equipment, and supplies; the use of tightly fitting can covers and, possibly, parchment liners; and the scrubbing of utensils and equipment immediately before use to remove settled particles. The filtration of air in dairy establishments would be another possible preventive measure, and general good housekeeping and sanitation practices would minimize possible contamination from air-borne particles. The fundamental control of the more objectionable extraneous matter lies in the elimination of the rodents and insects themselves.

SUMMARY AND CONCLUSIONS

1. A method was developed and used for the examination of air for some types of extraneous matter. It provided for the filtration of air through a standard 1¼-inch lintine milk sediment test disc at the average rate of 26.1 cu. ft. per minute. A sedimentation method also was used in which large petri plates were exposed for 2-hour periods.

2. Examinations for air-borne material were made at creameries, cream stations, and farms during late summer and fall of the year. The extraneous matter encountered was grouped into the following nine types: feather parts, rodent hairs, other hairs, insect parts, vegetable matter, cloth fibers, sand and clay, coal dust, and unidentified.

3. Sand and clay, coal dust, and cloth fibers were the most common types of identified material obtained by the air examination methods used. Vegetable matter, insects parts, and hairs other than rodent types were obtained less frequently. Generally, rodent hairs and feather parts were found the least frequently. All of these types of material, however, were obtained from the air around creameries, cream stations, and dairy farms by both of the methods used for examination. The results obtained by one procedure generally supported those obtained by the other.

4. Most of the particles encountered were small, requiring the use of the wide field microscope for identification. The larger particles encountered commonly were of vegetable origin, and were obtained most frequently in dry, windy weather, particularly by the sedimentation procedure.

5. The main significance of the investigation is that some of the same types of material reported in sediment tests of dairy products were found to be air-borne around dairy establishments.

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SOURCES OF VARIATION IN THE BIRTH WEIGHT OF HOLSTEIN-FRIESIAN CALVES[1]

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Differences between calves in size at birth sometimes are used as an indication of differences between them in vigor, potential growth rate, and mature size. If variations in the size of calves at birth are to be considered in selection or mating plans, knowledge of the relative importance of the various sources of this variability can be used for standardizing the nongenetic influences, predicting the progress from selection, and estimating the effects of inbreeding on birth weight.

REVIEW OF LITERATURE

Sex of calf, calving sequence, weight of dam, length of gestation, breed, and heritable difference within breeds have been reported as factors influencing birth weights (5, 6, 7, 8, 11). These investigations showed significantly lighter average birth weights for calves from immature dams. Knapp and coworkers (7) in their analysis of birth weights of 98 beef Shorthorn and dairy Shorthorn cattle found a correlation of 0.55 between birth weight and length of gestation, 0.21 between birth weight and calving sequence, and 0.22 between birth weight and weight of dam. Thirty-eight per cent of the variation in birth weights could be ascribed to length of gestation, calving sequence, and weight of dam combined. Season of calving had little or no influence on birth weight. To estimate heritability an intra-class correlation of 0 17 between calves out of the same dam was calculated from the data for beef Shorthorn and dairy Shorthorn calves. The relationship between these calves would be 25 per cent (half-sibs) or more; hence the heritability could be estimated at 0.68 or less. The heritability of birth weight in range cattle has been estimated at 0.2 to 0.4 by Knapp and Nordskog (8). Eckles (5) indicated that maternal size influences birth weight of calves over and above the effect of calving sequence. Woodward and Graves (16) found that intense inbreeding (25 to 50 per cent by Wright's coefficient) in both Guernsey and Holstein-Friesian grade cattle resulted in lighter calves at birth. Bartlett and coworkers (1), on the other hand, found no significant differences between the birth weights of inbred and outbred Holstein-Friesian cattle in their experiment. A 10 per cent difference in birth weight between inbred (16 per cent) and outbred Holstein-

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Friesian calves by the same sire, in favor of the outbreds, has been reported by Dickerson (3) on early data drawn from the same herds reported here.

EXPERIMENTAL

The data for this study were obtained between 1937 and 1945 from three herds of Holstein-Friesian cattle owned by the Wisconsin State Department of Public Welfare. The birth weights of 395 male and 399 female calves born as singles were obtained during the period of the experiment. All calves born as twins have been omitted from the study. The number and average birth weight of the outbred (F_x less than 0.06—Wright's (20) coefficient) calves have been summarized by herd, sex, and calving sequence of their dam in table 1. The inbreds ($F_x \gtreqless 0.06$) are classified similarly in table 2.

In both outbred and inbred groups the males were significantly heavier than the females, the average difference being 5.1 and 6.5 lbs., respectively, for all three herds. Both male and female calves from herds 1 and 3 were significantly heavier at birth than those from herd 2. Although the difference between the weights of the male calves in herds 1 and 3 was small for both inbreds and outbreds, the inbred female calves in herd 1 were 4.3 lbs. heavier than the inbreds in herd 3, a significant difference.

The effect of calving sequence of dam is clearly demonstrated in tables 1 and 2. The first calf heifers in general gave birth to lighter calves of both sexes. The sole exception appears to be the dams of the inbred males in herd 2, but the small number of individuals observed here probably accounts for the inconformity. Calving sequence apparently has no influence after the second calving.

Analysis of variance was carried out to test the significance and to determine the relative importance of various factors on birth weight. In order to estimate the variance attributable to the main effects and their interactions, the data were analyzed using the method of expected subclass numbers (13). This method is based on the hypothesis that the population subclass numbers are proportional or equal and that the disproportionate numbers in the sample merely are chance variations. The mean square within subclasses is used as the residual variance to test the significance of the various main effects and their interactions. The average birth weights of the calves for the three herds, the five calving sequences, and both sexes for all outbred and inbred calves, weighted according to expected numbers, are given in table 3.

Herd, Sex, and Calving Sequence

Herd, sex, calving sequence, and the interaction between herds and calving sequence (Herd × CS) were significant sources of variation in birth weight in the non-inbred group of calves. The Herd × CS mean square

TABLE 1

Number and mean birth weight of outbred calves according to herd, sex, and calving sequence of dam

Herd	1				2				3				Total			
Calving sequence	Male		Female		Male		Female		Male		Female		Male		Female	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean
		(lbs.)		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*
1	14	90.9	14	88.3	18	82.2	20	76.9	7	90.7	4	76.0	39	86.8	38	81.0
2	21	101.2	24	95.0	11	86.0	21	83.3	8	102.0	11	95.2	40	97.2	56	90.7
3	19	100.8	19	99.4	13	87.7	9	87.4	12	108.2	4	101.0	44	98.9	32	96.3
4	14	98.6	6	90.5	18	89.2	11	82.7	14	104.4	9	98.8	46	96.7	26	90.1
5 & over	12	104.9	11	96.9	22	87.7	18	87.2	24	101.3	28	96.2	58	96.9	57	93.5
Total ..	80	99.4	74	94.8	82	86.6	79	82.9	65	102.2	56	95.3	227	95.6	209	90.5

TABLE 2

Number and mean birth weight of inbred calves according to herd, sex, and calving sequence of dam

Herd . . .	1				2				3				Total			
Calving sequence	Male		Female		Male		Female		Male		Female		Male		Female	
	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean
		(lbs.)		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*		*(lbs.)*
1	27	85.8	23	84.4	7	80.7	9	67.2	27	80.5	47	77.3	61	82.9	79	78.2
2	8	98.2	2	87.5	6	82.5	11	74.5	28	93.9	27	88.8	42	93.1	40	84.9
3	3	91.3	2	93.0	9	80.0	10	77.5	20	96.6	24	85.2	32	91.4	36	83.5
4	2	96.5	3	104.0	2	80.0	3	83.3	11	90.7	14	88.4	15	90.1	20	90.0
5 & over	5	104.4	3	93.3	5	82.0	4	82.5	8	97.5	8	86.5	18	95.1	15	86.8
Total .	45	90.9	33	87.7	29	81.0	37	75.1	94	90.6	120	83.4	168	89.0	190	82.5

TABLE 3

Mean birth weight for herd, sex, and calving sequence for all outbred and inbred calves, weighted according to expected numbers

Item	Outbreds	Inbreds
	(lbs.)	*(lbs.)*
Herds		
1	97.2	90.9
2	85.3	77.2
3	97.6	86.1
Sexes		
Male	95.4	88.4
Female	90.3	83.0
Calving sequence		
1	84.3	79.2
2	93.4	89.1
3	96.8	88.7
4	93.5	90.4
5	95.4	91.4
Total	92.9	85.5

measures the variation in the differences between calving sequence from herd to herd. The other first order interactions and the second order interactions were not significant.

The variance components of the mean squares in table 4 can be separated according to the sources of the differences between individual birth weights.

A = variance within individual subclasses
B = additional variance due to Herd × Sex × Calving Sequence interaction
C = additional variance due to Sex × Calving Sequence interaction
D = additional variance due to Herd × Calving Sequence interaction
E = additional variance due to Herd × Sex interaction
F = additional variance between Calving Sequence
G = additional variance between sexes
H = additional variance between herds

Inasmuch as the mean squares for the Sex × *CS* and the Herd × Sex × *CS* were smaller than the within subclass mean square, *B* and *C* were assumed to be zero (table 4). Estimates of the other variance components are as follows:

Variance	*Source*	*Amount*	*Per cent of total*
A	Within	103.5	54.6
D	Herd × *CS*	3.9	2.1
E	Herd × Sex	1.1	0.6
F	*CS*	19.4	10.2
G	Sex	12.0	6.3
H	Herd	49.7	26.2
	Total	189.6	100.0

The birth weights of the inbred calves have been analyzed similarly. The main effects all were significant sources of variation, while none of

TABLE 4

Analysis of variance of birth weights of outbred calves (expected subclass number technique)

Source of variation	Degrees of freedom	Mean square	Variance components of mean square*
Herd	2	7499.5†	$1.19 A + 14.95 B + 29.85 D + 72.27 E + 144.29 H$
Sex	1	2818.0†	$1.17 A + 15.22 B + 43.40 C + 73.59 E + 217.60 G$
Calving Sequence (*CS*)	4	1912.2†	$1.17 A + 14.64 B + 43.30 C + 29.23 D + 86.50 F$
Herd × Sex	2	206.0	$1.19 A + 14.90 B + 72.02 E$
Herd × *CS*	8	234.4‡	$1.19 A + 14.33 B + 28.61 D$
Sex × *CS*	4	64.2	$1.18 A + 14.59 B + 43.15 C$
Herd × Sex × *CS*	8	76.0	$1.20 A + 14.28 B$
Within subclasses	406	103.5	A

* The method used in computing the coefficients of the components of variance when the numbers in the subclasses are disproportionate was obtained from S. L. Crump, Associate Professor, Statistical Laboratory, Iowa State College, Ames, Iowa.

† $P < 0.01$.

‡ $P < 0.05 > 0.01$.

the interaction mean squares were large enough to be significant, although C and D were positive. The components of variance were estimated as follows:

Variance	*Source*	*Amount*	*Per cent of total*
A	Within	104.8	56.0
C	Sex × *CS*	0.8	0.4
D	Herd × *CS*	0.6	0.3
F	*CS*	33.7	18.0
G	Sex	14.2	7.6
H	Herd	33.2	17.7
	Total	187.3	100.0

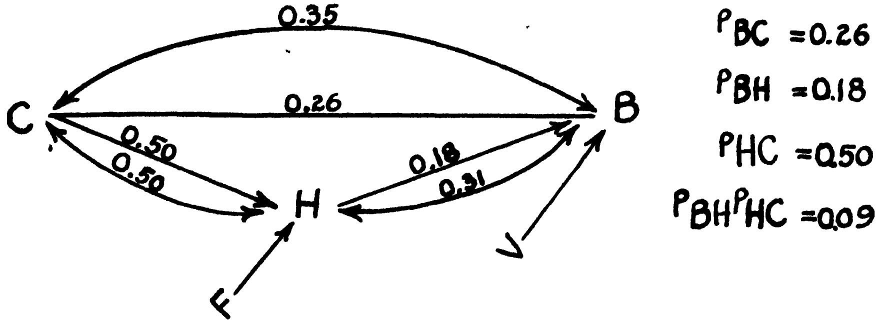
In these data herd, sex, and calving sequence of dam accounted for approximately 44 per cent of the total variation in birth weights as compared to 45 per cent in the non-inbred group.

Size of Dam

Measurements of heart girth taken approximately 1 month following calving were available as an indication of body weight (2) on the dams of 728 calves which were weighed at birth. The analysis of covariance gave the following intra-herd, intra-sex correlations between dam's heart girth (H), calving sequence of dam (C), and birth weight of calf (B) based on 722 degrees of freedom:

$$r_{BH} = 0.31$$
$$r_{BC} = 0.35$$
$$r_{CH} = 0.50$$

The method of path coefficients (18, 21) was used to determine the relative importance of dam's heart girth and calving sequence as causes of variation in birth weights of calves. The relationships may be diagramed logically as follows:



In this diagram the straight lines represent standard partial regression coefficients (path coefficients) and the curved lines designate correlation coefficients. The letter *F* represents all other unanalyzed causes of variation in heart girth independent of calving sequence, and *V* represents all other sources of variation in birth weight independent of both calving sequence and heart girth.

The correlation between birth weight and calving sequence has been divided into two components. One, $p_{BC} = 0.26$, measures the influence of calving sequence on birth weight independent of its association with size of dam. The other, $p_{BH}p_{HC} = 0.09$, represents the effect of calving sequence on birth weight through its association with heart girth. The results here indicate that the effects of calving sequence of dam, independent of her size, may be slightly more important than her size (heart girth) as a source of variation in birth weight. The indication of curvilinearity for the relationship between *C* and *B* means that this form of analysis gives only a rough approximation of the relative importance of these factors.

Year and Season

The effects of year and season on birth weights were analyzed separately for each sire to avoid any confounding effects of sire and time trend. The method of fitting constants (13) was used in the analysis because of the disproportionate subclass numbers and the absence of data in some subclasses. This method is described as being based on the assumption of zero interaction; nevertheless, the method can be used to test the significance and to estimate the size of interactions.

Birth weights (adjusted to a mature dam and female equivalent) of the offspring of six sires in two herds were tested for seasonal and yearly effects. The analyses of the sires' offspring in herd 3 are presented in table 5. There were no significant differences between years or seasons.

TABLE 5

Analysis of variance of birth weights of calves by sires in herd 3 (method of fitting constants)

Source of variation	Sires					
	R		C		S	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Years	3	197	6	56	6	52
Seasons	3	83	3	49	3	64
Years × Seasons	4	212	13	90	11	103
Within subclasses	41	132	80	109	62	113

The number of offspring per sire in herd 2 was small; hence it did not seem feasible to make a detailed analysis using the method of fitting constants. The regular analysis of variance did not indicate any significant yearly or seasonal effects (table 6).

Heredity

The correlations between the birth weights of full-sibs, paternal half-sibs, and maternal half-sibs, as well as the intra-sire regression of offspring's birth weight on dam's birth weight, were used in the present study as a basis for calculating heritability of birth weight. The birth weight of each calf was standardized to a mature dam basis (second calving sequence and over) and to a female calf equivalent within each herd. The adjustment factors for each herd were computed from data in tables 1 and 2.

Correlations between the birth weights of paternal half-sibs were derived from the analysis of variance of birth weights in which the variances in each herd were divided into portions due to sire differences and to differences between the offspring of the same sire. If A represents the variance between members of a progeny group and B is the additional variance between non-half-sibs, the correlation between offspring by the same sire corresponds to the fraction which B is of $A+B$. This is an estimate of the reduction in

TABLE 6

Analysis of variance of birth weights of calves by sires in herd 2

Source of variation	Sires					
	7003		301		353	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Years	4	3	4	112	2	150
Seasons	3	45	3	35	3	29
Within subclasses	36	58	45	50	26	108

total variance that should result if all the calves were by the same sire but out of different dams.

If, however, the genetic relationship within groups is larger than that of paternal half-sibs, A is expected to be smaller and the fraction $\frac{B}{A+B}$ larger than that for paternal half-sibs. Conversely, if there is any relationship between groups, both B and $\frac{B}{A+B}$ should be decreased. In either case, $\frac{B}{A+B}$ represents the average correlation between individuals within the groups.

TABLE 7

Variances and intra-class correlations between birth weights of half- and full-sibs within herds

Approximate relationship	Herd	No. of calves	No. of progeny groups	Variance*		$\frac{B}{A+B}$	Prob.
				A	B		
Paternal half-sibs	1	224	14	119.0	13.3	0.10	$\cong 0.05$
	2	209	6	80.3	16.5	0.17	< 0.01
	3	332	6	118.8	23.6	0.17	< 0.01
	Total	765	26	108.3	13.2	0.11	< 0.01
Maternal half-sibs	1	138	63	132.1	4.6	0.03	> 0.05
	2	165	74	92.4	− 6.2	− 0.07	> 0.05
	3	246	118	103.4	28.5	0.22	< 0.01
	Total	549	255	107.0	12.4	0.10	< 0.05
Full-sibs	1	44	22	67.9	− 4.7	− 0.07	> 0.05
	2	77	35	68.6	46.9	0.41	< 0.01
	3	160	64	70.0	36.1	0.34	< 0.01
	Total	281	121	69.3	33.0	0.32	< 0.01

* A = Variance within sires, within dams, or within sets of parents.
B = Additional variance due to differences between sires, between dams, or between sets of parents.

The number of individuals, the number of sire groups, the A and B portions of the variance, and the intra-sire correlations $\left(\frac{B}{A+B}\right)$ in each herd are listed in table 7. The correlations between the birth weights of paternal half-sibs within each herd were significant and the differences between the correlations for different herds were not significant. When the data for the three herds were pooled, the intra-sire correlation was 0.11.

The deduced intra-dam correlations for maternal half-sibs and full-sibs also are given in table 7. The correlation of 0.22 between maternal half-sibs in herd 3 was the only one of the three which was significant, but figures for the three herds did not differ significantly. When the data on the 255 dams were pooled on the intra-herd basis, the correlation between maternal half-sibs was 0.10, not significantly different from 0.11 calculated for paternal half-sibs. The correlations between full-sibs were significant for two of the herds, but they are subject to a large sampling error because of the small number of full-sib comparisons.

The analysis of covariance was used to obtain the intra-sire regression of offspring's birth weight on dam's birth weight. This regression of offspring on dam within groups of offspring by the same sire should eliminate most of the environmental contributions to the correlation between parent and offspring. Regression is to be preferred to correlation in such data because the dams may have been a selected group with correspondingly lower variance and consequently lower correlation but unaffected regression.

The average birth weights and the intra-sire correlations and regressions of offspring's birth weight on dam's weight by herds are given in table 8.

TABLE 8

Average birth weights, and intra-sire correlations and intra-sire regressions of offspring's birth weight on dam's birth weight by herds

Herd	No. of pairs	No. of sires	Offspring av. birth weight	Dam's av. birth weight	Intra-sire		
					r	b	Prob.
			(*lbs.*)	(*lbs.*)			
1	20	5	95.0	105.1	0.52	0.64	$\cong 0.03$
2	34	3	81.0	87.6	0.66	0.61	< 0.01
3	107	5	91.0	92.4	0.24	0.30	< 0.01
Total . . .	161	13	. .		0.34	0.40	< 0.01

The variations in regression coefficients from herd to herd were not significant. When the data for all herds were pooled, the intra-sire regression coefficient was 0.40, while the correlation coefficient was somewhat smaller, $r = 0.34$, due to the lower variance of the birth weights of the dams.

The different estimates of the heritability of birth weights are given in table 9 by herds. The paternal half-sib correlation in a random breeding population would be expected to contain one-fourth of the additive genetic variance, some (probably one-sixteenth or less) of the epistatic variance, plus the environmental portion of the variance times the correlation between the environments of paternal half-sibs. The maternal half-sibs correlation is composed of essentially the same sources of genetic variance as paternal half-sibs, but it may be larger due to the maternal half-sibs having been exposed to a more similar prenatal environment. The correlation between full-sibs should contain one-half of the additive genetic variance, about one-fourth of the dominance deviations, some (probably one-fourth) of the epistatic deviation, and any common prenatal environmental effects; the intra-sire regression of offspring on dam should contain one-half of the additive genetic variance plus some (probably one-fourth) of the epistatic effects.

Non-random matings would tend to make the preceding estimates of

TABLE 9

Estimates of heritability (h^2) of birth weights

Derivation of h^2*	Herd			Total
	1	2	3	
3.17 × paternal half-sib correlation	0.32	0.54	0.54	0.35
3.19 × maternal half-sib correlation	0.10	.	0.70	0.32
1.88 × full-sib correlation		0.77	0.64	0.60
2.00 × intra-sire regression offspring on dam	1.28	1.22	0.60	0.80

* The correlation between two offspring by the same sire is:

$$r_{oo} = \frac{h^2(1 + 2r_{sd} + r_{dd})}{4(1 + r_{sd/2})} + e^2 r_{ee}$$

between two offspring by the same dam is:

$$r_{oo} = \frac{h^2(1 + 2r_{sd} + r_{ss})}{4(1 + r_{sd/2})} + e^2 r_{ee}$$

between full-sibs:

$$r_{oo} = \frac{h^2(1 + r_{sd})}{2(1 + r_{sd/2})} + e^2 r_{ee}$$

where r_{dd} is the correlation between genotypes of the mates of the sire, r_{sd} is the genotypic correlation between sire and dam, r_{ss} is the correlation between genotypes of the mates of the dam, e is the path for environmental factors common to the offspring, and r_{ee} is the correlation between the environments affecting the sibs (19). The values for r_{sd}, r_{dd}, and r_{ss} computed from these data were 0.130, 0.084, and 0.074, respectively. No estimates were made of e or r_{ee}.

heritability too high except for the intra-sire regression coefficients, which are not affected by deviations from random matings (9).

The average of the estimates of h^2 for birth weight in table 9 is 0.60, with a 95 per cent fiducial range of 0.50 to 0.70. Apparently between 50 and 70 per cent of the variation in birth weight of these calves was heritable after adjustment for sex of calf and calving sequence of dam.

Inbreeding

There were a total of 654 calves which had been weighed at birth, and whose dams had been measured after one or more calvings. These calves were sired by 21 bulls in the three herds. The inbreeding coefficients ranged

TABLE 10

Analysis of covariance of birth weights (b), inbreeding coefficients (i), and dam's mature heart girth (h)

Source of variation	Degrees of freedom	Correlations			Partial regression
		r_{bi}	r_{ih}	r_{bh}	$b_{bi.h}$
Total	653	−0.125*	....	....	....
Between sires	20	0.018	....	....	....
Within sires	633	−0.181*	0.141*	0.099†	−0.280*

* $P < 0.01$.
† $P < 0.05 > 0.01$.

from zero (318 calves) to 0.375—227 calves were inbred between 0.06 and 0.17; 55 had a coefficient between 0.18 and 0.25; while the remaining 54 were inbred higher than 0.25. The average inbreeding of the offspring by each sire ranged from 0.03 to 0.17.

The average effect of inbreeding on birth weight can be expressed by the intra-sire partial regression of birth weight on inbreeding percentage after adjustment for the linear regression of birth weight on dam's average mature heart girth dimension (table 10). The non-significant correlation of birth weight and coefficient of inbreeding between sires indicates that average birth weight of a sire's progeny was not associated with the average inbreeding of the group, but the negative correlation and partial regression within sires shows that of the individuals in a sire group the inbred calves tended to be lighter at birth. The variation between sires in the influence of inbreeding on the birth weight of their calves is shown in table 11. These partial regression coefficients differed significantly between sires, ranging from – 0.823 to + 1.090. Three coefficients were statistically significant; the others were no larger than expected from sampling errors.

TABLE 11

Number of offspring, average inbreeding (i), average birth weight (b), average of dam's mature heart girth (h), and the partial regression of birth weight on inbreeding holding dam's mature heart girth constant for 21 sires in three herds

Sire	No. of offspring	Av. inbreeding	Av. birth weight	Av. mature heart girth of dam	Partial regression $b_{bi.h}$
			(lbs.)	*(cm.)*	
S	95	0.03	98.5	195	– 0.110
C	118	0.09	89.9	193	– 0.223
R	51	0.13	90.2	192	– 0.571*
J	11	0.12	92.3	194	– 0.088
E	42	0.17	87.0	188	+ 0.139
U	12	0.13	91.7	189	+ 0.399
353	34	0.03	81.2	189	– 0.191
405	38	0.05	78.1	192	– 0.823†
301	57	0.03	84.4	190	– 0.073
7003	47	0.08	86.2	188	– 0.168
427	16	0.03	82.1	194	+ 0.483
1217	20	0.07	93.7	196	– 0.142
15	13	0.07	96.7	198	– 0.377
M	21	0.10	99.8	195	– 0.464†
1220	9	0.07	95.3	195	– 0.532
1192	16	0.08	101.2	195	– 0.380
1146	23	0.03	97.4	193	+ 1.090
1172	9	0.12	102.7	193	– 0.733
1221	8	0.13	88.1	190	– 0.517
1250	8	0.10	103.5	190	– 0.328
1274	6	0.07	100.5	186	+ 0.716
Total	633				– 0.280*

* $P < 0.01$.
† $P < 0.05 > 0.01$.

DISCUSSION

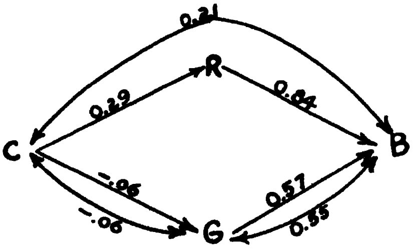
The study of the influence of herd, sex, calving sequence of dam, and dam's size (heart girth) showed significant contributions of each to the total variability in birth weight. The findings of heavier male than female calves, lighter calves from first calvings, and heavier calves from larger dams agree with other published results.

Calving sequence of dam, independent of her size, was found to be slightly more important as a source of variation in birth weight than was her size (heart girth). Both factors probably influence birth weight through their effect either on rate of intra-uterine growth or on the length of gestation period. The records analyzed in this study did not include lengths of gestation period, but Knapp *et al.* (7) reported correlations between calving sequence (C), weight of dam (W), length of gestation period (G), and birth weight (B) as follows:

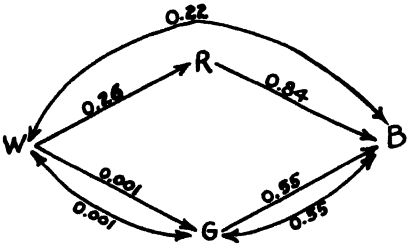
$$r_{CB} = 0.21 \qquad r_{CW} = 0.31$$
$$r_{WB} = 0.22 \qquad r_{CG} = 0.06$$
$$r_{GB} = 0.55 \qquad r_{WG} = 0.001$$

These correlations have been used in the following path coefficient scheme to estimate the paths through which calving sequence and weight of dam probably influenced birth weight. As before, the curved lines represent correlation, and the straight lines, path coefficients. Rate of growth (R) is represented as an alternative to length of gestation period (G) as an intermediate step between calving sequence (C) and birth weight (B). As pointed out by Wright (21), "Birth weight (B) is completely determined (in the mathematical sense rather than causally) by the prenatal growth curve and the age at which it is interrupted by birth." The correlation coefficients, in terms of their component path coefficients, furnish three equations; the formula for complete determination provides another. Solution of these four equations leads to the values given in the diagram and to the following values for the two routes from calving sequence to birth weight: $p_{BR}p_{RC} = 0.244$ $p_{BG}p_{GC} = -0.034$

Similarly for weight (W) of dam: $p_{BR}p_{RW} = 0.220$ $p_{BG}p_{GW} = 0.001$



PATHS FROM CALVING SEQUENCE TO BIRTH WEIGHT



PATHS FROM WEIGHT OF DAM TO BIRTH WEIGHT

Calving sequence and weight of dam seem to have exerted most of their influence on birth weight through their effect on intra-uterine growth rate. ($p_{BR}p_{RC} = 0.244$ *vs.* $p_{BG}p_{GC} = -0.034$, and $p_{BR}p_{RW} = 0.220$ *vs.* $p_{BG}p_{GW} = 0.001$) This is in agreement with the work of Wright (19) on guinea pigs and Lush *et al.* (10) on pigs in the sense that factors affecting birth weight, such as size of litter, calving sequence, and dam's weight, seem to operate more by affecting rate of growth rather than by interrupting prenatal growth by birth at an earlier or later time.

Correlations between related individuals were used to estimate heritability of birth weight. These correlations include differing amounts of covariance from additive gene effects, dominance deviations, epistasis, and environmental factors. The small numbers of observations and the restricted number of relationships available meant that the relative importance of each of these components could not be estimated. It would appear, however, that the degree of heritability of birth weight from additive gene effects in these data is about 50 to 70 per cent.

The influence of inbreeding on birth weight was small, but highly significant. The reduction in birth weight amounted to approximately 1 lb. for every 4 per cent increase in inbreeding. There was, however, a large amount of variation between sires in the influence of inbreeding on birth weights. This variation between sires could be caused by differences in the average birth weight transmitted by the different sires. Inbred calves from the sires transmitting heavy birth weights would tend to be heavier than outbred calves by the same sire, simply because the inbred calves are more closely related to the sire. Conversely, inbred calves would be lighter than outbred calves at birth for sires transmitting light birth weight. Thus differences in the average birth weight inheritance of the sire would cause the intra-sire regression of birth weight on inbreeding to vary from sire to sire more than would be expected by chance alone. The average intra-sire regression of birth weights on inbreeding represents the effect of increased homozygosity and indicates that dominance and perhaps gene interaction are involved in the action of the genes which influence birth weight.

SUMMARY

Data on Holstein-Friesian calves in three unrelated herds were analyzed to determine some of the causes of variation in birth weight and to compare the birth weight of inbred and outbred offspring by the same sire.

The mean birth weights of 794 calves were 92.9 lbs. and 85.5 lbs. for the outbreds and inbreds, respectively. Male calves averaged 5.2 lbs. heavier than females and calves from "first calf" heifers were approximately 10 lbs. lighter than were calves from dams of later calving sequence. Herd differences accounted for about 22 per cent, sex for 7 per cent, and calving sequence for 14 per cent of the total variation in birth weight. Season and

year had little or no effect on birth weight. Approximately 60 per cent of the remaining variation in birth weight was due to the genotype of the calf.

Birth weight declined an average of 0.28 lb. for each increase of one per cent inbreeding, with size of dam constant. The inbreeding effect varied significantly from sire to sire, apparently due to differences in the average birth weight transmitted by different sires. The inbred calves of sires transmitting heavy birth weights tended to be heavier than the outbred calves because of having more of their sires' genes, which cancels, at least in part, the reduction in birth weight from increased homozygosity.

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CHANGES IN MILK PRODUCTS "SHAM FED" TO CALVES.
I. EFFECTS OF VOLUME OF MILK FED[1]

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The functions of the secretions of the oral cavity and of the esophageal passage in the utilization of milk by the young calf have not been explored extensively. Preliminary observations of the effects of sham-feeding on the properties of whole milk suggested that the modifications were of such a nature as to expedite subsequent digestion (8). It was postulated that the magnitude of the alteration of the milk would be affected by the quantity of saliva contacted during ingestion. Furthermore, the amount of oral fluids mixed with the milk would depend on the rate of consumption and on the volume and constancy of salivary flow.

The paucity of information on the rôle of saliva in the physiology of the nutrition of the calf prompted a study of the pre-gastric changes of milk ingested by calves of suckling age. Since the quantity of milk consumed and the system of feeding it are considered to be factors affecting the well-being of the calf, the effects of these factors and associated reactions on the degree of modification of the milk prior to its entering the stomach were investigated.

EXPERIMENTAL

Methods and Procedures

Feeding procedures. Four rumen-fistulated calves (1), two Holsteins and two Guernseys, between 5 and 6 months of age were sham fed (8) mixed-herd pasteurized milk testing 4.2 per cent fat. In each of four series of feeding trials two calves of the same breed were sham fed consecutively, one from a nipple feeder and the other from an open pail. The system of feeding each calf was alternated from trial to trial to compensate for differences arising from individuality (8). In order to reduce the number of factors affecting the volume and the concentration of the oral fluids, the calves were changed to different surroundings shortly before feeding to minimize the effects of possible conditioned reflexes. A further standard measure followed was withholding feed and water for a period of 6 to 8 hours after the regular morning feeding. At the end of this time, the individual calves were sham fed 25 lbs. of milk (approximately five times the volume that would

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be fed normally) in 5-lb. increments. The rate of consumption of each successive unit of milk was determined by means of a stopwatch. There were momentary interruptions between consumption of the consecutive units to add milk to the feeder pails and to change collecting receptacles.

Determination of the properties of the milk. Sampling and analyses of the individual sham-fed units were initiated 20 minutes after the feeding. The properties determined on the milk samples, sham-fed and unconsumed (control), included: (a) lipolysis, (b) hydrogen-ion concentration, (c) rate of rennet coagulation, and (d) fat percentage.

a. *Lipolytic activity.* The degree of lipolysis was determined in all the samples by the steam distillation procedure described by Roahen and Sommer (6). For comparative purposes, lipolysis in samples from half the trials, two obtained using each feeding system, was estimated by determining the acid number (2) of the lipid material removed by Soxhlet extraction of 45 to 50 g. of milk dehydrated by means of calcium sulfate.

b. *Hydrogen-ion concentration.* This was measured by means of a Beckman glass electrode pH meter. Frequent cleaning of the electrodes by immersion in dilute hydrochloric acid was necessary to avoid erratic readings.

c. *Rennet coagulation time.* The method used was essentially the same as outlined by Sommer and Matsen (7). The only modification of the procedure was the employment of a temperature of 38° C. (approximately the body temperature of cattle) instead of the recommended 30° C. in the tempering bath.

d. *Fat percentage.* The concentration of fat in the various samples of milk was determined by the aforementioned Soxhlet extraction procedure. Since the ether-extracted material included practically all the fat constituents and saliva is assumed to be practically free of ether-soluble substances, the changes in the "fat percentages" in the sham-fed units were used as a basis for calculating the degree of dilution resulting from sham feeding.

RESULTS

The curves in figure 1 represent averages of data from a series of four trials in which each calf was sham fed once by each of the two systems, pail and nipple. The results presented in figure 2 are from a series of two trials, involving one calf of each breed.

As previously noted (8), sham feeding perceptibly altered the properties of whole milk. Lipolysis apparently was the primary change as indicated by the titration values of fatty acids in the steam distillate (fig. 1A) and in the ether extracts (fig. 2A), both procedures yielding similar results. The presence of free fatty acids in the sham-fed samples was indicated further by the decreased pH values (fig. 1B). The increased rate of coagulation by rennet (fig. 1C) seemed to bear a positive relationship to the changes in acidity, though other factors probably were involved in accelerating this

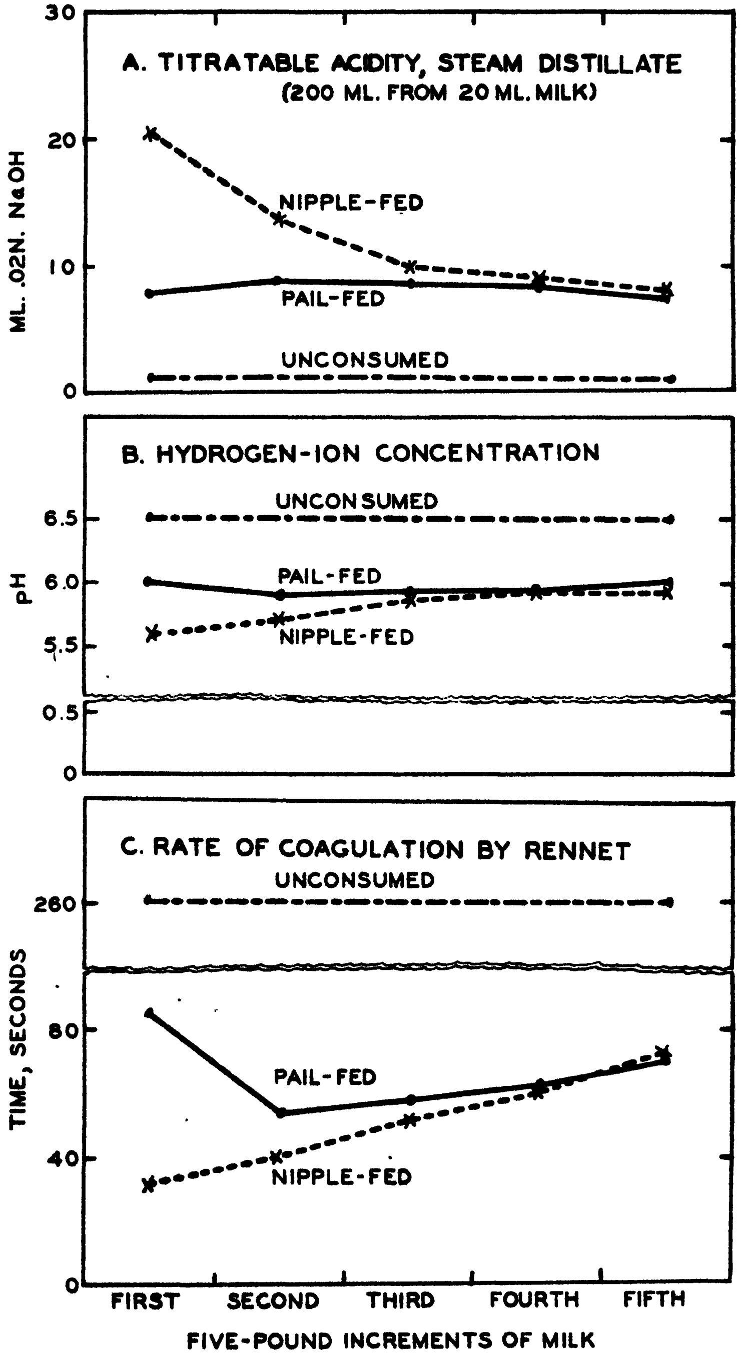

FIG. 1. Effect of volume of whole milk sham fed by open pail and nipple feeder, respectively, on the changes in acidity and in rate of rennet coagulation.

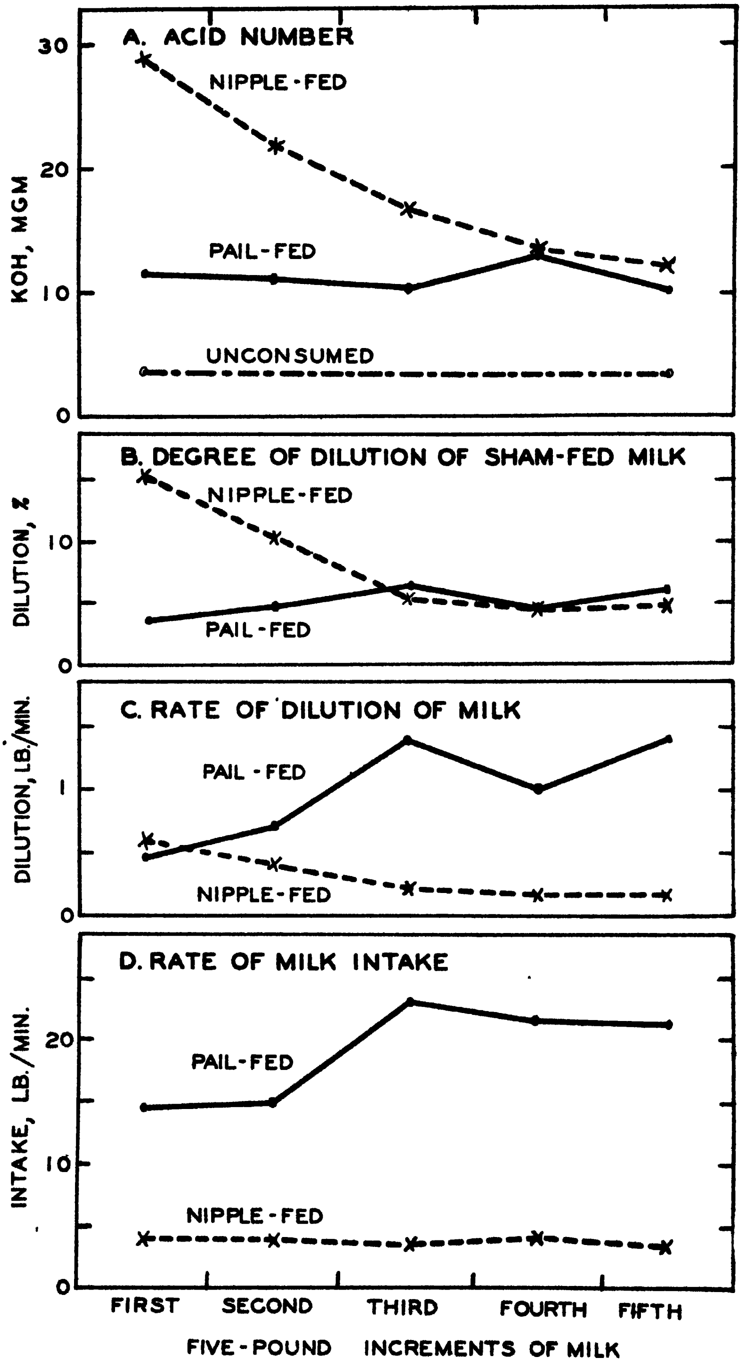

FIG. 2. Relation of changes in acidity in sham-fed whole milk to dilution in feeding and to rate of intake.

complex reaction. Moreover, the results are in accord with previous observations (8) showing that the degree of modification of the milk was greater when the nipple system of feeding was used than when the open pail was employed. This was especially marked in the first increments fed.

The system of feeding also was a factor modifying the effects of volume of milk consumed on the magnitude of the changes (fig. 1A, B and C and fig. 2A). When the milk was pail fed, the lipolysis as indicated by acidity measurements was essentially the same in all the 5-lb. units; when the milk was nipple fed, the degree of lipolysis was greatest in the first portions and least in the last, the reduction being most pronounced in the first two increments. After the consumption of the third 5-lb. unit, the extent of lipolysis tended to be similar for the two feeding systems. Though there was a general tendency for rennet coagulability to parallel the trends in acidity, the degree of change in rate of rennet coagulation from the first to the second increment of milk sham fed from the open pail was more marked than the corresponding change in acidity. The explanation for this pronounced acceleration of rennet coagulation is obscure.

The specific factors involved in the modification of the properties of milk in sham feeding are not clearly indicated. Indirect evidence implicating saliva and/or other oral fluids and esophageal secretions in the lipolytic activity was derived from the positive relationship between the volume of fluids mixed with the milk in feeding (fig. 2B) and the associated lipolysis (fig. 2A). In nipple feeding the average percentage dilution ranged from 15.3 for the first increment to 5.0 for the last, the trend revealing a close relationship with that for lipolysis. The degree of dilution and the extent of lipolysis in the first two increments of pail-fed milk were decidedly less than in the nipple-fed milk, but in subsequent units the magnitude of change from the two systems was similar. The range in average dilution of pail-fed milk was from 3.5 to 6.4 per cent, but the trends were erratic, tending to show a reciprocal relationship with the lipolytic changes. Since the variations in percentage dilution were within the probable experimental error of the analytical procedures, this tendency toward a negative relationship with lipolysis probably was fortuitous.

In comparing the results from the two systems of feeding, the rates of dilution in addition to degrees of dilution were considered. The rate of dilution (fig. 2C) presumably was determined by the volume of secretions with which the milk came in contact, and this appears to have been modified by the rate of intake (fig. 2D). The rate of consumption was slow and uniform from the nipple feeder but rapid and erratic from the open pail, showing a marked increase after the second increment. The intake by drinking was from four to six times faster than by sucking. When the milk was sham fed from the open pail, the total amount of 25 lbs. was consumed in 1.4 minutes, resulting in a calculated dilution of 1.3 lbs. The same dilution, by weight, resulted from the consumption of only 10 lbs. of milk from the nipple feeder but required 2.6 minutes. In spite of the divergent rates of dilution (fig. 2C) and of intake (fig. 2D) by the two feeding systems, the degree of dilution was essentially the same after the second 5-lb. increment.

DISCUSSION

Accrued evidence definitely indicates that marked lipolysis takes place in whole milk sham fed to calves ranging in age from 3 weeks (8) to 5 months. The sources of these lipolytic agents remain obscure. Previous observations (8) led to the postulation that the enzymes involved are glandular in origin. The close relationship between the degree of dilution and the magnitude of lipolysis, particularly in the nipple-fed milk, suggests that saliva is a vehicle and/or an activating medium for the specific agents. Since saliva is defined as the mixed secretion of the three pairs of main salivary glands and the numerous small glands in the mucous membrane of the mouth (3), the origin of the lipolytic constituents is indefinite.

Before saliva definitely can be implicated, additional evidence is necessary. If insalivation of milk is involved, as suggested, modification of the secretory processes should be reflected in lipolytic changes. Hence, a more direct approach through the study of the effects of various salivary stimulating and suppressing drugs on the degree of lipolysis in sham-fed milks is indicated. The lipolytic activity of the secretions from the major salivary glands also merits investigation.

If the quality and the amount of saliva secreted are adapted to the character of the food (3), the degree of dilution from sham feeding indicates that whole milk has the characteristics necessary to stimulate salivation. Though the specific glands involved are not indicated, Dukes (3) stated that in ruminants parotid secretion is continuous; however, Fomin (5) reported that milk usually depresses the activity of these glands in calves. Since the parotid glands are serous, the type that has enzyme-producing cells (3), the degree of lipolysis observed in the sham-fed milk suggests stimulation instead of suppression. The lipolytic agents, possibly, may be discharged from other sources.

If the assumption that the dilution is due primarily to saliva be correct, the rate of secretion varied with the system of feeding, with the volume of milk fed, and/or with time. When the milk was consumed from a nipple pail, the rate of intake from increment to increment was almost constant (fig. 2D); therefore, the degree and the rate of dilution (fig. 2B and C) should bear a close relationship to the rate of secretion. Thus it appears that as successive units were consumed by sucking, the volume of saliva decreased. To the contrary, feeding from an open pail resulted in increases in rate of dilution (fig. 2C) concomitant with accelerated intake (fig. 2D), thus suggesting increased salivation as additional amounts were consumed.

These results from the two systems of feeding appear to be discordant, but it is necessary to take cognizance of the time element.

On a time basis the first 5-lb. increment sucked from the nipple feeder corresponded to the entire 25 lbs. drunk from the open pail. This leads to the postulation that the initiation of ingestion of milk stimulates the rate of salivation, which increases to a maximum followed by a gradual decline to almost a constant flow. In accordance with this theory the peak salivation from sucking probably was attained either in the first increment or the early part of the second; whereas a crest of salivary flow during drinking presumably occurred in the third increment. Though there is some indication that sucking *per se* tends to stimulate salivation (8), this was not manifested in this study after consumption of the first unit of milk. It is possible that sucking stimulates the flow of saliva to the maximum quicker than does drinking.

In modern dairy husbandry practices a calf would not receive more than 10 lbs. of whole milk at one feeding. Since the differences between the two systems of feeding were most marked in the first two increments (10 lbs.) of sham-fed milk, theoretically the nipple feeder would have an advantage over the open pail. The increased dilution of milk by oral fluids, contrary to the results from aqueous dilution (4), seems to be associated with accelerated coagulation by rennet. It is possible that the degree of pre-gastric modification may be reflected in subsequent differences of digestion and utilization. The practical aspects of this problem merit further investigation.

SUMMARY

1. In a series of trials each of four calves was sham fed from nipple and open pails, respectively, 25 lbs. of whole milk in five successive 5-lb. units.

2. Marked lipolysis, as manifested by increased acidity and concomitant acceleration of coagulation by rennet, was observed in whole milk that was sham fed to the calves.

3. When the milk was sham fed from a nipple feeder, the alteration of the properties was greatest in the first portions fed and least in the last, but when fed from an open pail, the differences in magnitude of change in successive units were not marked except in rennet coagulability, which was most rapid in the second increment and less in subsequent units.

4. Though the degree of change in the first increments was more marked when consumed by sucking than by drinking, the alterations were similar for both systems of feeding after the third increment.

5. Lipolysis in the successive 5-lb. units closely paralleled the changes in degree of dilution, indicating that lipolytic agents were closely associated with the diluting medium.

6. The rate of intake from the open pail was from four to six times more rapid than from the nipple feeder, but the differences in rates of dilution

were less, being approximately the same in the first increment but diverging in subsequent units.

7. The system of feeding and/or the time factor affected the dilution of the milk as the volume of intake increased.

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STUDIES ON THE SUSCEPTIBILITY OF CERTAIN CHEESE CULTURES TO THE ACTION OF BACTERIOPHAGE[1, 2]

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Slow acid production during the manufacture of cheese has been encountered occasionally by many cheesemakers. In most of these instances the duration of the period of slow acid production was not long and the mere introduction of another culture or a modification of the method of culture preparation remedied the difficulty. Factors other than bacteriophage probably were responsible for the majority of these cases of slow acid production. Quite recently several outbreaks of slow acid production due to bacteriophage have occurred. During these outbreaks cheesemakers attempted to control the difficulty by the use of methods that had eliminated slow acid production when bacteriophage was not the causative factor. Generally, these control methods did not eliminate the slow acid production.

Since outbreaks of slow acid production definitely established as due to bacteriophage are of relatively recent origin in this country, few articles have been published and the literature from foreign countries where difficulties with bacteriophage have been experienced is not readily available to many cheese manufacturers.

HISTORICAL

The importance of bacteriophage in relation to vitality of cheese cultures was brought out by Whitehead and Cox (8). These investigators stated that, from a practical point of view, it is necessary to find some method of eliminating bacteriophage or employing an organism that is immune to its action. The isolation of cultures immune to bacteriophage appeared to be the best solution to the problem. Attempts were made to produce bacteriophage-resistant cheese cultures by isolating strains of *Streptococcus cremoris* from a culture after it had been attacked by bacteriophage and after secondary growth had appeared (9). One culture prepared in this manner was resistant to low concentrations of the bacteriophage strain with which it was treated, but high concentrations would attack it. The process of immunization was repeated with another bacteriophage preparation and a third culture isolated. It soon became apparent that this process of immunization was not permanent. In addition to the appearance of more bacteriophages, the supposedly immune strains gradually lost their resistance to the bacteriophage with which they had been treated during the course of their isolation.

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Whitehead and Hunter (10) noted that a culture of streptococci which was attacked by bacteriophage ultimately showed growth with continued incubation. The streptococci which finally caused a clotting of the milk culture, although indistinguishable in most respects from the original strain, had a partial or complete resistance to the bacteriophage. Colonies grown from the resistant culture gave both lysogenic and non-lysogenic variants, which were both weak and strong acid producers in milk. These authors had hoped that the cultures, once having been resistant to bacteriophage, would be immune to sudden failure under commercial conditions. However, such cultures were just as susceptible to bacteriophage attack as the original culture.

Nelson *et al.* (6) found that strains of *Streptococcus lactis* isolated from milk or whey that had soured naturally were not inhibited by any of the available bacteria-free filtrates containing bacteriophages which were active against other organisms of this group. However, they state that strains of bacteriophage which are active against these non-sensitive strains of *S. lactis* might be found if a larger number of bacteriophage strains from a greater range of sources was used.

Whitehead and Hunter (11) proposed the use of a heavy inoculation of the bulk culture (1.0–1.6 per cent) for eliminating typical bacteriophage failures in cheese plants. They advanced the theory that the effect of the heavy inoculation in eliminating the spontaneous appearance of bacteriophage was due to the more rapid growth of the streptococci when they were transferred to each new batch of milk, *i.e.*, to the shortening of the lag period. They believed that bacteriophage tended to appear whenever the lag period was prolonged as a result of light inoculation, low incubation temperature, and aeration or aging of the milk medium. Later, these investigators (12) attempted to eliminate culture failures due to bacteriophage by three methods: (a) destruction of air-borne bacteriophage, (b) protection of cultures in the factory, and (c) preparation of the culture in a separate building. The destruction of air-borne bacteriophage by spray treatment with disinfectants was exceedingly difficult, since the disinfectant had to be distributed in a very fine state to be effective, and reinfection was continuous. Protection of the bulk culture by means of an airtight container, fitted with a small cotton-plugged opening through which the milk could be inoculated, limited the air-borne bacteriophage to an amount below that necessary to cause difficulty. The use of a separate building some distance from the cheese factory for the preparation of cultures appeared to be the only completely satisfactory means for eliminating trouble.

Anderson and Meanwell (1) state that many cheesemakers have adopted the policy of adding large quantities (up to 4 per cent) of culture to the milk intended for cheese in order to overcome slow acid production. This method frequently overcame the trouble, but manufacture of a good quality cheese by this method was difficult. The use of another culture usually gave temporary or complete relief of slow acid production for the remainder of the season. These investigators isolated lactic streptococci from sour milk samples obtained from different parts of the country and used these freshly isolated cultures in combination with commercial cultures. The introduction of the recently isolated strains into the culture combination was effective in overcoming slow acid production for a short time, but after a period of 10 days a condition resembling complete "pack up" (no acid production) was experienced. Experiments and experiences with single-strain cultures demonstrated the possible danger of their introduction into commercial plants. Reliance on multiple-strain cultures seemed more desirable, since the presence of a bacteriophage active against a single culture in a combination of cultures results in a condition of slowness rather than "pack up". A bacteriophage detection test based on the effect of bacteriophage in reducing the activity of the culture was used. When this test was used at a creamery for an entire season, no serious outbreak of slowness occurred. Since all cultures condemned by the bacteriophage detection test were discontinued immediately, it was difficult to produce absolute proof that the successful acid production of the cultures was due to the adoption of this system of control, but a study of the records provided direct evidence in support of this view.

Nichols (7) states that the secret of bacteriophage control is to insure that the bulk culture is free from bacteriophage contamination. When the bulk culture was free of bacteriophage, it was possible to make cheese even though bacteriophage was present in the cheese vats. However, when bacteriophage was present and the cheese vats were filled more than once during the day, it was not always possible to control slowness completely because of the increase in bacteriophage concentration during the day. Methods suggested for the protection of cultures from bacteriophage included destruction of bacteriophage in the factory, especially in the air, and preventing contamination of the milk, cheese vats, and other equipment. The author recommends that several cultures should be held in reserve and that these reserve cultures be selected so that no culture in the collection is susceptible to a bacteriophage attacking any of the others. The use of different cultures about which nothing was known was considered a dangerous practice.

METHODS

The methods employed for the preparation of bacteria-free filtrates from cheese cultures and whey, isolation of *S. lactis* from cheese cultures, formation of bacteriophage plaques, and sensitivity tests have been outlined previously (3).

Cultures employed in cheese manufacture. Cultures 99, HP, 146, 122, and N were from the regular collection maintained in the Dairy Industry

Department, Iowa State College. Culture W4 was a recent isolation from sour cream. Cultures 99, HP, and N were multiple-strain cultures, and 146, 122, and W4 were single-strain cultures.

Propagation of mother cultures. The mother cultures were propagated in the same manner previously described (2). These cultures were propagated in a laboratory located on the second floor of the Dairy Industry Building. The cheese laboratory was located on the basement floor and some distance to the rear of the laboratory used for inoculation and preparation of the mother cultures.

Preparation of bulk culture. Skimmed milk was heated to 200° F. in a 30 gallon stainless steel Cherry-Burrell univat. The milk was held at this temperature for 1 hour, cooled to 70° F., and inoculated with 0.5 per cent mother culture. Incubation of the bulk culture was for 16 hours at 70° F. The vat in which the bulk culture was prepared was located in one of the cheese-manufacturing laboratories. Cheese was not being made regularly in this particular laboratory but it was being made in an adjoining laboratory.

Purification of bacteria-free filtrates. Single-plaque isolations were made from petri dishes using as substrate cultures of *S. lactis* that were sensitive to the non-purified bacteria-free filtrates. A single isolated plaque appearing on the petri dish was touched with a sterile transfer needle and then transferred to a bottle containing sterile milk. The bottle was inoculated with a sensitive culture and incubated for 24 to 48 hours at 30° C. After the incubation period a bacteria-free filtrate was prepared. Sensitivity tests were made on this filtrate using cultures of known bacteriophage sensitivity. A portion of the filtrate (recently prepared from the single plaque isolation) was added to another bottle of sterile milk and inoculated with small quantities of several cultures of known bacteriophage sensitivity. After incubation for 24 to 48 hours at 30° C., another bacteria-free filtrate was prepared and used for the production of bacteriophage plaques. Another isolated plaque was picked from a petri dish and a bacteria-free filtrate prepared from it. If the two filtrates prepared by single plaque isolations showed the same bacteriophage sensitivity patterns, they were considered to be pure bacteriophage strains.

EXPERIMENTAL

When an outbreak of slow acid production due to bacteriophage occurred in the cheese-manufacturing laboratories of Iowa State College, an attempt was made to control the difficulty by using a culture which was not sensitive to the bacteriophage that had caused the previous culture to fail in acid production. At this time fourteen cultures were being transferred regularly in the Dairy Department. Several of these cultures were known to contain the same strain of *S. lactis*. The entire histories of certain of these cultures were known but with some of the others relatively little was known. Culture no. 1 had been used in the cheese laboratory for a considerable period of time before it suddenly failed to produce acid satisfactorily. A bacteria-free filtrate prepared from the whey of the vat in which slow acid production occurred showed the presence of bacteriophage which was active against all of the cultures being carried except five (99, HP, 146, 122, and N). The results obtained when these cultures were used in the manufacture of cheese are presented in table 1.

Culture 99 was the first culture used; it produced acid satisfactorily the the first day, and bacteria-free filtrates prepared from the bulk culture and whey from the vat in which this culture was used showed no bacteriophage activity toward any of the cultures. This culture also was used the following day and acid production by it was very slow. The presence of bacteriophage could be demonstrated in bacteria-free filtrates prepared from the bulk culture and whey. Both filtrates were active only against culture 99. The following day culture HP was used. Acid production by this culture was rapid and a bacteria-free filtrate prepared from the bulk culture showed no bacteriophage activity; the whey from the cheese vat showed bacteriophage activity against culture 99 but not against culture HP. The following day culture HP was used again. Acid development was slow and bacteria-free filtrates of the bulk culture and whey showed bacteriophage activity against culture HP. The bacteria-free filtrate of whey also showed the presence of bacteriophage active against culture 99.

TABLE 1

Susceptibility of cheese cultures to the action of bacteriophage when used in a cheese plant experiencing difficulty with bacteriophage

Date (1945)	Culture employed in manufacture of cheese	Rate of acid production during manufacture of cheese	Presence (+) or absence (−) of bacteriophage in bulk culture*	Presence or absence of bacteriophage in a bacteria-free filtrate of whey from the cheese vat when tested against cultures of *S. lactis* isolated from culture					
				99	HP	146	122	N	W4
March 21	99	Satisfactory	−	−	−	−	−	−	−
March 22	99	Very slow	+	+	−	−	−	−	−
March 23	HP	Rapid	−	+	−	−	−	−	−
March 24	HP	Slow	+	+	+	−	−	−	−
March 25	146	Rapid	−	+	+	−	−	−	−
March 26	146	Slow	+	+	+	+	−	−	−
March 27	122	Rapid	−	+	+	+	+	−	−
March 28	122	Slow	+	+	+	+	+	−	−
March 30	N	Satisfactory	−	−	−	+	+	−	−
April 2	N	Satisfactory	−	+	+	+	+	+	−
April 4	N	Slow	+	+	+	+	+	+	−
April 16	W4	Rapid	−	−	−	−	−	−	−
July 12	W4	Very slow	+	−	−	−	−	−	+

* Bacteria-free filtrate tested against a series of cultures of *S. lactis* isolated from the mother culture used for inoculation of the bulk culture.

Culture 146 produced acid rapidly the first day it was used and on the second day produced acid slowly. On the first day of use, bacteriophage active against cultures 99 and HP was present in the whey, and on the second day, bacteriophage active against cultures 99, HP, and 146 was present in the whey. Bacteriophage active against culture 146 was not present in the bulk culture on the first day this culture was used, but it was present in the bulk culture on the second day. Culture 122 was used after culture 146 had failed to produce acid; it produced acid rapidly·the first day. The bulk culture was free of bacteriophage the first day, but a bacteria-free filtrate of the whey showed bacteriophage activity toward cultures 99, HP, 146, and 122. Acid production by culture 122 was slow the following day and bacteriophage active against this culture was detected in the bulk culture. A bacteria-free filtrate of the whey showed the presence of bactriophage active against cultures 99, HP, 146, and 122. Bacteriophage titres were determined on this sample of whey, using cultures of *S. lactis* of known bacteriophage sensitivity that were isolated from cultures 99, HP, 146, and 122. The titres were 10^{-1}, 10^{-1}, 10^{-2} and 10^{-9}, respectively.

No cheese was made on March 29, and on March 30 culture N was used. It produced acid satisfactorily the first day and no bacteriophage active against culture N was demonstrable in the bulk culture; a bacteria-free filtrate of the whey showed the presence of bacteriophage active against cultures 146 and 122. Cheese was not manufactured on March 31 or April 1, and culture N was used again on April 2. Acid production was satisfactory on April 2, and the bulk culture was free of bacteriophage active against culture N. However, the whey from the cheese vat contained bacteriophage active against cultures 99, HP, 146, 122, and N. No cheese was made on April 3, and on April 4 culture N produced acid very slowly. On this day bacteriophage active against culture N was detected in the bulk culture, and the whey from the cheese vat showed the presence of bacteriophage active against cultures 99, HP, 146, 122, and N. Bacteriophage titres on the bacteria-free filtrate of the whey using cultures of *S. lactis* of known bacteriophage sensitivity isolated from cultures 99, HP, 146, 122, and N were 10^{-7}, 10^{-8}, 10^{-8}, 10^{-7}, and 10^{-9}, respectively.

On April 16 culture W4 was used in the manufacture of cheese. This culture was a single strain of *S. lactis* isolated from a sample of sour cream. Culture W4 was not sensitive to any of the bacteriophage types that previously were encountered. This culture produced acid rapidly and consistently in cheese manufacture for a period of approximately 3 months before it failed to produce acid due to the effect of bacteriophage. Culture M1, also isolated from sour cream, produced acid satisfactorily for over 6 months and was discontinued in order to study other cultures.

Several investigators (1, 5) have stated that they prefer to use a multiple-strain cheese culture when difficulties are encountered with bacteriophage.

Since all strains hardly would be sensitive to bacteriophage, acid production, therefore, would continue at a slower rate rather than stop entirely, as would be expected with a cheese culture containing a single strain of *S. lactis*.

Culture no. 1, which was employed in the cheese laboratory at the time slow acid production first occurred, was a multiple-strain cheese culture. It appeared desirable to determine what proportion of the strains in this culture were sensitive to the bacteria-free filtrate, prepared from the whey of the vat in which slow acid production occurred, since acid production practically ceased during cheese manufacture. This cheese culture was plated, and after sufficient growth had occurred fifteen colonies were picked from a section of the petri dish. The results obtained when these cultures were tested for bacteriophage sensitivity, using the bacteria-free filtrate prepared from the whey of the vat in which slow acid production occurred, are presented as a portion of table 2. Only three of the fifteen cultures selected for study were resistant to the action of the bacteria-free whey filtrate.

TABLE 2

Separation of single bacteriophage strains from a bacteria-free filtrate composed of several bacteriophage strains

Streptococcus lactis culture no.	Activity of original whey filtrate	Activity of filtrates prepared by single-plaque isolations from the original filtrate			
		Type 10	Type 1	Type 4	Type 2
1	+	–	–	+	+
2	+	–	+	–	+
3	+	–	+	–	+
4	+	–	–	+	+
5	+	–	–	+	–
6	+	–	–	+	–
7	–	–	–	–	–
8	–	–	–	–	–
9	+	–	–	+	+
10	+	+	–	–	–
11	+	–	–	+	–
12	+	–	–	+	+
13	+	–	–	+	–
14	+	–	–	+	–
15	–	–	–	–	–

It also appeared feasible to determine whether the bacteriophage activity of the whey filtrate was due to one bacteriophage strain or whether this filtrate contained several bacteriophage strains. Single plaque isolations were made from petri dishes, using for plaque formation each of the strains of *S. lactis* which showed sensitivity toward the original whey filtrate. The data presented in table 2 show that four bacteriophage strains were present in the whey filtrate. One bacteriophage strain was active against only one of the fifteen *S. lactis* cultures, while the other bacteriophage strains were active against two, nine, and six of the fifteen *S. lactis* cultures. Since the purified bacteriophage filtrates exhibit distinct bacteriophage activity patterns, they are considered to be different bacteriophage types.

The fifteen cultures of *S. lactis* isolated from cheese culture no. 1 have been transferred regularly in tubes of litmus milk for a period of more than 2 years. These cultures have been tested for bacteriophage sensitivity against a number of bacteria-free filtrates prepared from whey or cheese culture obtained from plants experiencing difficulty with slow acid production in cheese manufacture. At the present time ten bacteriophage types which are active against some of these fifteen cultures have been isolated and purified. The activity patterns of these bacteriophage types are presented in table 3.

TABLE 3

Action of purified bacteria-free filtrates containing bacteriophage on cultures of S. lactis isolated from a multiple-strain cheese culture

Streptococcus lactis culture no.	Bacteriophage activity pattern no.									
	1	2	3	4	5	6	7	8	9	10
1	−	+	+	+	+	+	−	+	−	−
2	+	+	−	−	+	+	+	+	−	−
3	+	+	−	−	+	+	+	+	−	−
4	−	+	+	+	+	+	+	+	−	−
5	−	−	+	+	+	+	+	+	−	−
6	−	−	+	+	+	+	+	+	−	−
7	−	−	+	−	−	+	−	+	−	−
8	−	−	−	−	−	−	−	+	−	−
9	−	+	+	+	+	+	+	−	+	−
10	−	−	−	−	−	−	−	+	−	+
11	−	−	+	+	+	+	+	+	−	−
12	−	+	+	+	+	+	+	+	−	−
13	−	−	+	+	+	+	+	+	−	−
14	−	−	+	+	+	+	−	+	−	−
15	−	−	−	−	−	−	−	−	−	−

Bacteriophage types have been isolated which are active against fourteen of the fifteen cultures of *S. lactis*. Culture no. 15 is not sensitive to any of the bacteriophage types. *S. lactis* culture no. 8 is sensitive to only one type, culture no. 10 to two types, and culture no. 7 to three types of bacteriophage. The remainder of the cultures are sensitive to from five to seven bacteriophage types. On the basis of the bacteriophage activity patterns, it appears that the fifteen *S. lactis* cultures isolated from the multiple-strain cheese culture represent ten distinct strains of *S. lactis*. Cultures 2 and 3 have the same sensitivity pattern, as do cultures 4 and 12, and cultures 5, 6, 11, and 13. Cultures 1, 7, 8, 9, 10, 14, and 15 all are different.

DISCUSSION

In the trials involving the use of various cultures in the manufacture of cheese (table 1), slow acid production was not evident during the manufacture of cheese unless the bulk culture was contaminated with bacteriophage. These results tend to confirm the results of Nichols (7), who noted that when the bulk culture was free of bacteriophage, it was possible to make cheese even though bacteriophage was present in the cheese vats. When the bulk culture is free of bacteriophage, only slight contamination is expected to result from the equipment and air, provided that the equipment has been washed and sterilized properly. However, if the equipment is not washed and sterilized properly, sufficient contamination may result to cause slow acid production in the cheese vat. Johns and Katznelson (4) were able to cause complete cessation of acid development in a cheese vat by using a culture which was free of bacteriophage and contaminating the milk by placing some bacteriophage on a rake used for stirring.

Although the cultures selected for cheese manufacture were not sensitive to the bacteriophage types which previously had caused slow acid production, only 1 or 2 days were required for the culture to develop sensitivity toward bacteriophage. Cultures recently isolated from sour cream required a considerably longer period before they developed bacteriophage sensitivity.

When cheese was made on March 24, bacteriophage present in the whey was active against cultures 99 and HP. This does not indicate that cultures 99 and HP are sensitive to the same types of bacteriophage. Since culture 99 was used on 2 previous days and bacteriophage active against this culture was present, the bacteriophage active against culture 99 may have gained entrance to the vat from equipment or air contamination. On March 30, the bacteria-free filtrate prepared from the whey did not show bacteriophage activity against cultures 99 or HP. Probably sufficient time had elapsed to cause dilution of these bacteriophage types and they were not detected. The bacteriophage type which was active against culture N appeared to be active against all of the other cultures.

The results presented in table 2 show that three of the fifteen cultures studied were resistant to the bacteriophage type which caused the slow development of acid. By calculation it would be expected that one-fifth as much acid would be produced in the cheese vat; however, acid development practically ceased. It should be emphasized that all strains of *S. lactis* in a multiple-strain culture do not produce acid at the same rate. The cultures of *S. lactis* selected for study were obtained from a bacteriophage-free mother culture. The distribution of the various strains may be quite different in the bulk culture after it has been contaminated with bacteriophage.

Table 3 shows that one culture of *S. lactis* was resistant to all of the bacteriophage types isolated to date. Although the culture is resistant, it produces lactic acid very slowly and thus would not be suitable as a single-strain cheese culture.

SUMMARY

During an outbreak of slow acid production in cheese manufacture due to bacteriophage, an attempt was made to control the difficulty by the use of cultures that were not sensitive to the bacteriophage types responsible for the outbreak. These cultures had been carried in the regular culture collec-

tion. The five cultures selected for study developed sensitivity to bacteriophage after being used in the manufacture of cheese for 1 or 2 days.

One culture (W4) isolated from sour cream produced acid rapidly in cheese manufacture for approximately 3 months before it developed sensitivity toward bacteriophage. Culture M1, also isolated from sour cream, produced acid satisfactorily for more than 6 months; at the end of this period it was not sensitive to bacteriophage.

Multiple-strain cheese cultures may fail as completely as single-strain cultures to produce acid. Four distinct types of bacteriophage were recovered from a sample of whey taken from a vat in which a multiple-strain culture was used and in which there had been complete cessation of acid production.

Ten bacteriophage types which are active against one or more of fifteen cultures of *S. lactis* isolated from a multiple-strain cheese culture were isolated from various plants experiencing difficulty with slow acid production. The bacteriophage types were differentiated by means of their bacteriophage activity pattern.

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ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-SECOND ANNUAL MEETING

MANUFACTURING SECTION

M1 Application of the Phosphatase Test to Various Dairy Products.

GEORGE P. SANDERS AND OSCAR S. SAGER, Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

An improved modification of the phosphatase test, for application to Cheddar cheese and to fluid milk, was described by the writers in the November, 1946, issue of the JOURNAL OF DAIRY SCIENCE. As a result of further research, this test recently has been found reliable for detecting underpasteurization in cream, ice cream, sherbet, chocolate drink, butter, sweet buttermilk, cultured buttermilk, most of the principal varieties and kinds of cheese, and cheese whey.

The chemical principles of the test are the same for all dairy products. The optimal pH for the activity of the enzyme in the test has been found to be 10.0 to 10.05, and the optimal pH for development of indophenol blue color with a minimum of off-color interference has been found to be 9.3 to 9.4. Some of the different products have different buffering capacities, and the buffering capacities of the various cheeses increase during curing. Modifications, therefore, have been made in the concentration of the barium borate-hydroxide buffer to provide optimal enzymic activity in testing each product. Corresponding modifications have been made in the type and concentration of the zinc or zinc-copper protein precipitant in order to yield clear filtrates, promote complete color development, and minimize off-color interference.

The test is more sensitive than earlier tests and, by means of a dilution method used in developing and determining the amount of blue color, has been made quantitative for all the dairy products mentioned.

Production of phosphatase by microorganisms growing on the surface of some soft and semi-soft ripened cheeses—*e.g.*, Limburger, Liederkranz, and Camembert—has been encountered and this complicates an evaluation of the results of pasteurization. In most of these instances this effect has not been great enough to cause "false positive" results when the samples

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have been taken carefully from the center of the cheese by a method especially designed to prevent contamination of the sample. To date, no "false positives" have been encountered in testing samples of cream and butter in storage. All tested samples of process cheese and spreads made commercially have been found to be negative.

M2 Time-temperature Conditions Required to Inactivate Phosphatase in Different Dairy Products. George P. Sanders and Oscar S. Sager, Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

A laboratory pasteurizer was used, comprising a metal coil 30 feet long and ⅛-inch internal diameter with a metal holding chamber at the end. The coil was immersed in a water bath, the temperature of which was controlled thermostatically with a variation not greater than ± 0.1° F. In heating fluid products, a 25-ml. sample was forced into the coil under pressure. The temperature of the sample increased to that of the bath in not more than 4 seconds. The holding time at the bath temperature was controlled with an error not greater than ± 3 seconds. Cheese samples (3 g.) were ground and placed in heat-sealing metal foil envelopes, which were squeezed to a thickness of 1 mm., sealed, and immersed in the bath. The time required for the temperature of the sample to reach that of the bath at 150° F. was not more than 18 seconds, as determined with a thermocouple.

Phosphatase test data for samples heated at any specific temperature for various periods of time show that the rate of destruction of the enzyme by heat is very rapid at first and diminishes to a relatively very slow rate with time. The experimental data for phosphatase destruction show that, in tests on milk and other fluid dairy products, a straight line results when the logarithms of the times of heating are plotted against the corresponding temperatures.

Holding periods required in this test to reduce phosphatase activity to two units per 0.5 ml. of whole milk were: 37 minutes at 143°, 30 minutes at 143.6°, 24 seconds at 160°, and 15 seconds at 161.7° F., respectively. The temperature required to produce a negative phosphatase test in any given time generally was found to be about 0.7° lower for skim milk than for whole milk, about 0.7° higher for 20 and 40 per cent cream than for whole milk, about 4.5° higher for ice cream mix than for whole milk, and about 5.7° higher for sherbet than for whole milk. The time required, at 143° F., was about three times as long for ice cream mix as for whole milk.

Results of a large number of tests on milks heated under controlled conditions and on cheese made from milks similarly heated indicate that maximal values of two units per 0.5 ml. for milk and three units per 0.25 g. for most kinds of cheese are suitable as criteria for pasteurization. Phosphatase was inactivated at considerably lower temperatures and shorter holding times in Cheddar cheese than in milk—*e.g.*, at 130° F. in 13 minutes and at 140° F. in slightly less than 0.5 minute in cheese at pH 5.29. Mixing alkalies with the cheese to increase the pH had some effect in stabilizing the enzyme against heat. Adding emulsifiers had a greater effect, as the temperature required to produce a negative test in approximately 0.5 minute was 150° F. when 1.5 per cent anhydrous disodium phosphate was added and the pH of the mixture was 5.56.

M3 Determining the Germicidal Potency of Quaternary Ammonium Compounds and Their Use in Dairy Sanitation. W. S. Mueller, D. B. Seeley, and E. P. Larkin, University of Massachusetts.

A procedure for evaluating the germicidal properties of quaternary ammonium compounds is given. While the method basically is not new, it involves the use of newer procedures, such as the use of a hand homogenizer and the spectrophotometer. This method appears to give a truer picture of the germicidal potency under actual working conditions than is given by the phenol coefficient values as determined by the Food and Drug Administration method.

Organisms like *Escherichia coli, Staphylococcus aureus,* thermophilic types, and the vegetative cells of *Bacillus cereus* readily were killed by quaternaries. While quaternaries appeared to have no greater germicidal effect on spores of *B. cereus* and thermoduric types than older types of germicides, they were highly bacteriostatic to sporeformers.

Approximately 0.3 per cent of either cow manure or nonfat milk solids produced the first significant decrease in germicidal potency of a 200 p.p.m. quaternary solution. When this concentration of quaternary was used as an udder wash under normal conditions, approximately 30 to 40 cows could be washed before the germicidal potency of the solution decreased to a point where a fresh solution was needed. No chapping or cracking of udders, teats, or milkers' hands was noted when this concentration of quaternary was used.

Metal parts of dairy equipment, when properly washed, were effectively sanitized by the use of 200 p.p.m. of quaternary. However, as much as 400 p.p.m. was not effective in sanitizing the rubber inflation tubes of teat cups between each cow milked when the cups were dipped rapidly in and out of the solution. A quaternary spray treatment for milk cans showed a significant reduction in counts. Dual-purpose powders such as cleaner-sanitizer combinations appear to have considerable merit.

M4 Effectiveness of Hypochlorite and Quaternary Ammonium Germicides in Destroying *Streptococcus agalactiae* in a Mastitis Sani-

tation Procedure. K. R. Spurgeon, P. R. Elliker, W. J. Harper, and J. R. Froedge, Department of Dairy Husbandry, Purdue University.

A study was made of the comparative bactericidal activity, in various concentrations, of a number of germicides representing both hypochlorite and quaternary ammonium compounds. The most rapidly acting sodium hypochlorite and quaternary ammonium compounds were selected for further comparison of effectiveness in destroying *Streptococcus agalactiae* on teats and *Str. agalactiae* and *Staphylococcus aureus* on milking machine teat cups. Preliminary trials with inhibitors to stop germicidal action after prescribed periods of exposure indicated that sterile skim milk effectively neutralized the small residual of germicide remaining in experiments with teats and teat cups with both types of compounds.

Germicides applied to teats which had been inoculated with a suspension of *Str. agalactiae* did not eliminate all organisms present, but did destroy about 90 per cent of those that would have remained after ordinary rinsing of the teats with a non-germicidal solution. There was no pronounced difference in the effectiveness of the two types of germicides. Effectiveness of germicides was reduced markedly when old teat cups with surface breaks and cracks were used. *Staph. aureus* exhibited definitely greater resistance to both types of germicides than *Str. agalactiae* when inoculated onto teat cups.

The effectiveness of both germicides was increased by prerinsing teat cups in water to remove organic matter before treating with germicidal solution. The rinsing effect of the germicidal solution also removed large numbers of organisms from teat cups. Dipping teat cups in a germicidal solution at 51.7° C. was slightly more effective than dipping in a solution at 21.1° C.

Because of the possibility of contaminating teat cups from the rinse water commonly used to remove milk solids before dipping them in a germicidal solution, trials were carried out to determine the advantage of using a germicidal solution for this prerinse. Successive dipping in two germicidal solutions in most cases was more effective in destroying organisms on teat cups than dipping in plain water followed by a germicidal solution.

When the numbers of *Str. agalactiae* in sterile milk inoculated onto teat cups were high, (*e.g.*, more than 200 per teat cup), a number of cells usually survived successive dipping of the teat cups in a germicidal solution at 21.1° C., followed by one at 51.7° C. However, when the number of organisms inoculated onto teat cups was low (*e.g.*, less than 50 per teat cup), it usually was not possible to recover *Str. agalactiae* by means of sterile swabs after teat cups were treated with two successive dippings of either hypochlorite or quaternary ammonium germicidal solution.

M5 The Resazurin Reductase Test Using Prepared Sterile Dry Vials.

N. S. Golding, State College of Washington.

The use of the resazurin reductase test for the bacterial quality of milk has increased steadily in recent years. Its great advantage over the methylene blue test is its greater flexibility and speed. The operation of the resazurin test in a factory where no trained bacteriological worker is available meets with certain difficulties associated not with the basic principle of the test but rather with its operation.

By experimentation a method using 3-dr. screwcapped vials, each containing 0.05 mg. of dry resazurin, was developed to overcome most of the objections to the mechanics of the test under plant conditions. The method of preparing the vials is as follows: (a) Arrange the clean 3-dr. vials in shelf metal trays 10 vials wide. (b) Sterilize in steam at 15 lb. for 5 minutes. (c) With a dispenser consisting of 10 1-ml. hypodermic syringes, fill 10 vials at a time with 1 ml. each of a 0.005 per cent resazurin solution prepared with sterile distilled water. (d) Return the trays to the autoclave and sterilize at 5 lb. steam pressure for 5 minutes. (e) Change valves on autoclave to operate as a vacuum drying oven, and inspect the vials in each tray over a white surface. (f) Complete the drying in vacuum and allow the vials to cool. (g) Cap with dry sterile caps and pack 1 gross per box.

The capped vials containing sterile dried resazurin have been kept more than 6 months and may be used at any time during that period. Any deterioration of the resazurin in these vials has not been noted and even considerable exposure to light does not appear to affect the resazurin. Using a modified rack for the vials, a Marschall methylene blue constant temperature water bath, and a suitable sampling dipper, all the resazurin test is done directly on the receiving platform as the milk is received.

It is not claimed that the resazurin test using prepared sterile dry vials is in any way basically different from the widely used standard method for operating this test. However, after using vials containing dry sterile resazurin in a factory during the last 2 years, it definitely is concluded that the method is rapid, sound, and economical, and gives more uniform results. Where operators of the test have little or no bacteriological and chemical training, the results still should be reliable.

M6 Some Observations on the Inversion of Sucrose by Invertase.

L. E. Mull and L. R. Arrington, Florida Agricultural Experiment Station.

An experiment was conducted to determine some of the factors affecting the inversion of sucrose by invertase. The variable factors studied were concentration of sucrose solution, pH of sucrose solution, concentration of enzyme, temperature, and time required for inversion. The effects of these factors were measured by determining the amount of sucrose inverted. In order to ascertain whether or not these factors might affect the amount of dextrose and levulose formed, the percentage of each of these also was determined.

Sucrose solutions at concentrations of 55 and 65 per cent were acted upon by invertase concentrations of 15, 20, 30, and 40 mg. per 1,000 g. of sucrose for periods up to 156 hours. The pH values tried were approximately 3.5, 4.5, 5.5, and 6.5. The temperatures employed were 45, 55, and 65° C.

From the data obtained, the following observations were made: (a) The sucrose concentration of 55 per cent was inverted at a more rapid rate than the concentration of 65 per cent. (b) Hydrogen-ion concentration in the range of pH 4.5–5.5 permitted the most rapid inversion, while at pH 3.5 inversion progressed more slowly and the rate of inversion was slowest at pH 6.5. (c) The rate of inversion increased proportionately as enzyme concentrations were increased from 15 to 40 mg. per 1,000 g. of sucrose. (d) The optimum temperature for inversion appeared to vary with pH. At 65° C. pH 4.5–5.5 resulted in the most rapid rate of inversion. (e) Inversion was most rapid during the first portion of the inversion period and steadily declined as inversion continued. Inversion was not considered complete until about 156 hours. (f) The ratio of dextrose to levulose varied very little from 1.2 to 1.

M7 Observations on the Microscopic Analysis of Raw and Pasteurized Milk. G. H. Watrous, Jr. and F. J. Doan, Department of Dairy Husbandry, Pennsylvania State College.

Bacterial clump counts obtained with a modified microscopic procedure and an altered stain and staining technique were compared with standard plate counts, using 32° F. incubation, on 406 samples of milk before and after laboratory pasteurization. The samples studied represented producers shipments as received at a milk plant during all seasons of the year. The direct microscopic modifications consisted of the use of less methylene blue and acetic acid in Newman-Lampert formula no. 2 stain, coupled with slight changes in the preparation of the stained smear, and the use of a "strip counting" technique similar to that advocated by Levowitz. With these alterations, more certain recognition and counting of bacteria and bacterial clumps was possible.

With raw milk the results obtained indicate a relationship approaching a 1:1 ratio between bacteria counts obtained with this direct microscopic procedure and the standard plate method, the correlation being closer and more definite with high count milk than with low count milk, as would be expected.

There is no significant relationship between the microscopic clump count employed and the standard plate count of bacteria in fresh laboratory-pasteurized milk. Non-viable organisms apparently are capable of stain retention to a varying degree, undoubtedly influenced by the microflora of the individual sample.

Microscopic clump counts on fresh laboratory-pasteurized milk are considerably lower than those obtained prior to pasteurization, indicating that many cells destroyed by pasteurization are not capable of being stained. Little correlation exists between these counts on low count milk samples, but the relationship improves as the bacterial population increases. In practically all cases where the microscopic count of the raw milk sample is objectionably high, a similar count after pasteurization also is higher than can be considered satisfactory, indicating that the microscopic analysis of pasteurized milk, by the procedures used, has value in detecting bacterial inferiority in the raw milk used for pasteurization.

M8 An Activity Test for Cheddar and Cottage Cheese Starters. B. E. HORRALL AND P. R. ELLIKER, Department of Dairy Husbandry, Purdue University.

Studies on slow acid development in the manufacture of Cheddar cheese demonstrated the necessity in a plant starter program for a simple, accurate test to determine the activity of starter cultures under conditions simulating those in the cheese vat. The following method was developed to fulfill these requirements under practical conditions.

The milk was prepared by reconstituting high grade, spray-dried, nonfat milk solids in distilled water at the rate of 10 g. of milk solids per 90 ml. of water. This milk was sterilized in Erlenmeyer flasks at 15 lb. pressure for 10 min. Ten-milliter quantities of the reconstituted skim milk were pipetted into sterile screw-top test tubes and adjusted to 37.7° C. in a water bath. Each tube was inoculated with 0.3 ml. of the starter culture to be tested and incubated at 37.7° C. for 3.5 hr. At the end of the incubation period the entire contents of the tube, together with 5 ml. of distilled water used to rinse the tube, were titrated with tenth normal sodium hydroxide to a faint pink color, using phenolphthalein as indicator. The results were recorded as per cent lactic acid. This figure was termed the activity factor of the culture.

The data obtained indicated that in most cases low activity in starter cultures could be detected by this method before it became evident in the form of slow acid development in the cheese vat. Starter cultures that developed sufficient acid to yield a titratable acidity of 0.40 per cent or higher in the activity test resulted in cultures that were active in the cheese vat, providing bacteriophage, overheating in the vat, poor milk quality, or other abnormal factors were eliminated. Starter cultures with an activity factor of 0.30 to 0.35 were found to develop acid very slowly in the cheese vat.

A starter with an activity factor of less than 0.30 invariably developed little or no acid during the cheese manufacturing process.

This test or an earlier modification of it has been used daily for more than 2 years in four plants and for lesser periods in several others. It has proved a valuable aid in reducing incidence of slow acid production in the Cheddar cheesemaking process.

M9 Special Cultures for Manufacture of Blue Cheese from Pasteurized Milk.

C. E. PARMELEE AND F. E. NELSON, Dairy Industry Section, Iowa Agricultural Experiment Station.

A study was made of some possible means by which Blue cheese could be made from pasteurized milk and still develop the flavor characteristic of Blue cheese made from raw milk.

The addition of cultures of the lipolytic organisms *Achromobacter lipolyticum, Alcaligenes lipolyticus,* and *Pseudomonas fragi* to milk did not increase fat breakdown and flavor development in Blue cheese but sometimes resulted in unnatural, musty, and unclean flavors. Cultures of *Mycotorula lipolytica* added to pasteurized milk increased fat hydrolysis and improved the flavor of Blue cheese. The amount of improvement varied with different strains of the organism.

Eight strains of mold of the *Penicillium roqueforti* type were used in Blue cheese made from pasteurized milk. No strain was found to be especially suited to that type of cheese.

A mold-enzyme preparation, made by the growth of *P. roqueforti* on a modified Czapek's medium low in carbohydrate and high in butterfat, was added to the milk for Blue cheese. From 0.05 to 0.25 per cent of the preparation resulted in a desirable level of fat hydrolysis and flavor development in the cheese after curing for 12 weeks. Some strains of mold were more suitable for making this preparation than were others.

Total volatile acidity was found to be quite closely correlated to flavor score of the cheese. Cheese with total volatile acidities in the range in which 30 to 55 ml. of 0.1 N sodium hydroxide were necessary to titrate the volatile acids in the first 1,000 ml. of steam distillate from 200 g. of cheese generally had the highest flavor score.

M10 Lipase Production by *Mycotorula lipolytica.*

I. I. PETERS AND F. E. NELSON, Dairy Industry Section, Iowa Agricultural Experiment Station.

The production of lipase by *M. lipolytica* was enhanced by growing the organism at 30° C. in nutrient broth containing 0.05 per cent glucose and buffered at pH 4.5 with disodium phosphate and citric acid (0.225m).

Lipase activity was determined by adding 1 ml. of culture or cell-free filtrate to 50 ml. of an emulsion of 5 per cent butterfat in 0.25 per cent agar (containing 1:1500 formaldehyde) and incubating at 37° C. for 48 hours. To a 10-g. portion of incubated emulsion was added 25 ml. of neutral 95 per cent ethyl alcohol and 25 ml. diethyl ether, and the mixture titrated with *N*/20 potassium hydroxide in methyl alcohol, using phenophthalein as indicator. The lipase activity was expressed in acid degrees.

The yeast lipase was active over the pH range from 4.0 to 8.0, with the optimum at pH 6.2–6.5. When incubation periods extended beyond 48 hours, incubation temperatures of 28–33° C. resulted in greatest activity. Higher incubation temperatures resulted in a gradual inactivation of the enzyme, which was very pronounced at 48° C. and above.

The lipase-active substance was concentrated successfully by lyophilizing the culture, by use of the foam-adsorption procedure at pH 4.0, and by salting out with saturated ammonium sulfate. Salting out at 5° C. resulted in a more active precipitate than at 25° C.

Blue cheese made from pasteurized, homogenized milk to which was added cell-free filtrate of 2-day-old cultures resulted in greater fat hydrolysis and better cheese flavor than the uninoculated control. Lipase added in quantities to give volatile acidity titration values of 40–50 ml. *N*/10 sodium hydroxide per 200 g. of steam-distilled 12-week-old cheese was found most satisfactory. Higher values resulted in an excessively sharp and soapy taste.

The lipase of *M. lipolytica* offers possibilities for use in the production of Blue cheese from pasteurized milk.

M11 The Vapor Pressure-Moisture Relationships of Dry Milk Products.

R. W. KUNKEL AND S. T. COULTER, Dairy Division, University of Minnesota.

The water content-relative vapor pressure relationships of dry milk solids-not-fat, dry whole milk, dry ice cream mix, and various spray dried systems of milk constituents were studied. The relative vapor pressure of water over these products plotted against the water content gives S-shaped sorption isotherms such as are typical of a wide variety of materials.

Other work at this Station has demonstrated certain relationships that exist between the water content and the rates of deterioration during storage of dry whole milk and dry ice cream mix. Correlation of these data with data obtained on relative vapor pressure demonstrates that the log of the rate of reaction, with respect to the production of acid ferricyanide reducing substances and carbon dioxide, is a straight line function of the relative vapor pressure.

M12 Some Factors Influencing Spray Drier Design and Operation.

ARNOLD KITZES AND S. T. COULTER, Dairy Division, University of Minnesota.

Data showing the relationship between the humidity of the inlet air to the drier and the moisture content of the powder have been secured. The moisture content of the powder increases with increase in the inlet air humidity at any given rate of milk feed. The capacity of a drier to produce powder of a given moisture content is influenced materially by the humidity of the inlet air.

The vapor pressure-moisture relationships of dry milk at various temperatures have been determined. Attempts have been made to ascertain the drying rate of the milk particle. Data available at present indicate that the vapor pressure of the exit air from the drier always is lower than that of the water associated with the milk powder particle.

M13 Changes Produced in Milk on Heating.

H. A. HARLAND, R. JENNESS, AND S. T. COULTER, Divisions of Dairy Husbandry and Agricultural Biochemistry, University of Minnesota.

The effect of relatively long-time, high-temperature treatment of milk and milk components on the color and chemical properties has been studied in an attempt to elucidate the mechanism of the browning reaction of milk products.

Systems included in this work, in addition to skim milk, were buffered aqueous systems of lactose, caseinate, serum proteins, lactose-caseinate, lactose-serum proteins, lactose-caseinate-serum proteins, and caseinate-serum proteins. The heat treatments consisted of refluxing the systems in an oil bath. Aliquots were taken for analysis initially and after 1, 3, 6, and 12 hours of heating. The following determinations were made on the heated materials: lactose polarimetrically and by two reducing methods, acid ferricyanide reducing substance, pH, amino nitrogen, and fluorescence.

There was a lack of agreement among the methods for lactose when used on the heated products. Large losses of lactose were observed polarimetrically and small losses by the chloramine-T method. Values higher than the initial ones were obtained with the alkaline ferricyanide method.

The heat treatments resulted in small increases in the acid ferricyanide reducing substances of caseinate systems, but in very much larger increases when lactose was present. High reducing values are characteristic of those systems having a brown color. Thiamin disulfide reducing substances were highest in systems containing only proteins. Lactose containing systems yielded little or none of these substances.

Decomposition of lactose largely is responsible for the gradual decrease in pH of milk during heat treatment.

The amino nitrogen of systems containing serum proteins in the absence of caseinate decreased with heat treatment. There was no change in amino nitrogen in systems containing caseinate and lactose, but heating caseinate and buffer in absence of lactose resulted in a definite increase in amino nitrogen.

Heat treatment of lactose or caseinate systems resulted in increases in fluorescence of an acetone-water extract; much larger increases in fluorescence were observed on similar extracts of lactose-caseinate systems. Ten per cent KCl extracts of protein systems indicated the development of fluorescent substances during heat treatment.

The colors developed by the systems during heat treatment were recorded photographically. All of the systems containing lactose showed some degree of browning. Lactose-caseinate systems were of the darkest color, while caseinate alone acquired a slight but definite pink coloration. Serum proteins appear to have little effect on the formation of brown color in milk.

M14 Some Changes in Dry Whole Milk during Storage.

R. JENNESS, S. T. COULTER, H. A. HARLAND, AND L. K. CROWE, Divisions of Agricultural Biochemistry and Dairy Husbandry, University of Minnesota.

The effects of storage on dry whole milk have been studied in experiments designed to differentiate the respective rôles of temperature, oxygen level, and moisture level. Further experiments have been made on storage of dried simplified systems of milk constituents in an effort to determine which of them participate in the reactions that occur during storage. The progress of the deteriorative processes was followed by measurements of flavor, solubility, reducing capacity, fluorescence, browning, carbon dioxide production, oxygen utilization, and the concentrations of hydrogen ions, lactose, and amino nitrogen.

In addition to the tallowy flavor which develops only in the presence of adequate supplies of oxygen, two distinct stale flavors developing in gas-packed powder at different moisture levels have been observed. For a given powder at constant temperature, a linear relation holds between the log of rate of over-all loss of flavor score and moisture content. This rate differs from powder to powder and is accelerated greatly by increasing the storage temperature. The rates of change of solubility, reducing capacity (to acid ferricyanide), fluorescence, browning, carbon dioxide production, pH, and lactose concentration all are dependent on moisture level and temperature but not on oxygen level. Indications are that the reactions producing these changes involve lactose and protein. While considerable decreases in lactose concentration (as determined polarimetrically) occur during storage, particularly at high temperature and high moisture, no loss in amino nitrogen was found under the same conditions.

M15 The Relation of Ascorbic Acid to the Keeping Quality of Dry Whole Milk.

S. T. Coulter and R. Jenness, Divisions of Dairy Husbandry and Agricultural Biochemistry, University of Minnesota.

Ascorbic acid has been reported to be an essential link in the chain of reactions by which oxidized flavors develop in liquid milk. The rôle of ascorbic acid in the loss in flavor quality of dry whole milk has been investigated, using simplified systems as well as spray-dried whole milk. The simplified systems consisted of spray-dried mixtures of calcium phosphocaseinate and butterfat, both with and without ascorbic acid. In each of eight different trials a quantity of whole milk was split into three lots, one of which served as the control. In the second lot sufficient hydrogen peroxide was added before preheating to oxidize the ascorbic acid, and in the third lot the ascorbic acid was oxidized with hydrogen peroxide before preheating and 16 mg. of ascorbic acid per 100 g. of solids added to the milk after preheating. All lots were condensed in stainless steel equipment and then spray dried. The preheating was at each of the following temperatures for two trials: 63, 68, 74, and 79° C.

Tallowiness developed at an earlier date in the air-packed simplified systems containing ascorbic acid and became more intense than in those not containing ascorbic acid. Tallowiness did not develop in the nitrogen-packed simplified systems. The air-packed control samples of dry whole milk deteriorated in flavor quality at a significantly faster rate than the samples from which the ascorbic acid had been oxidized prior to preheating. There was no significant difference in the rates of flavor deterioration of the samples from which the ascorbic acid had been oxidized and those to which ascorbic acid was added after preheating. All of the air-packed samples after 12 weeks of storage at 37° C. had a tallowy flavor to a greater or less degree. The nitrogen-packed samples showed no significant differences in keeping quality, none being judged tallowy after 12 weeks of storage at 37° C.

M16 Factors Affecting the Ease of Reconstitution of Milk Powders.

U. S. Ashworth and H. A. Bendixen, Washington Agricultural Experiment Stations.

The ease of dispersion of whole milk powder is an important consideration to the consumer. A good whole milk powder was found to disperse completely to a concentration of 12.5 per cent total solids at a temperature of 68° F., with 20 back-and-forth excursions of about 1 foot during a period of 7 seconds. The physical state of the casein is the factor limiting the rate of dispersion. Precondensing to 40 per cent total solids before spray drying produced a powder which redispersed much more rapidly than did one made from the same milk precondensed to 20 per cent total solids. Preheating temperatures of 160 to 180° F. for periods of 10 minutes had no appreciable effect on the rate of dispersion of the powders.

Increasing the temperature of the water increased the rate of dispersion up to a certain temperature and then caused it to decrease. Apparently this decrease is caused by denaturing action of the warm water on the casein. Consequently, there is an optimum temperature of reconstitution. The effect of manufacturing methods on this optimum temperature will be presented.

Contact with water at 68° F. for periods up to 15 minutes adversely affects the reconstitution rate for the most rapidly dispersible powders. The amount of undispersed residue left after 7 seconds of manual shaking is approximately proportional to the time of standing in contact with water before agitation. Increasing the temperature of the water held in contact with the powder decreases the rate of dispersion.

M17 The Use of Nonfat Dry Milk Solids for Mother and Batch Starters.

B. E. HORRALL AND P. R. ELLIKER, Department of Dairy Husbandry, Purdue University.

Preliminary investigations on slow acid development in Cheddar cheese manufacture indicated that starter cultures varied in activity from day to day even though the milk used for the cultures was obtained from the same herd. Trials were carried out to compare uniformity and activity of *Streptococcus lactis* cheese cultures propagated in selected herd milk with reconstituted skim milk prepared from representative lots of high grade, spray, nonfat dry milk solids. The reconstituted skim milk contained 10 per cent milk solids.

Cultures carried in the reconstituted skim milk were more uniform in activity from day to day than duplicate cultures carried in the selected herd milk. Single and multiple strain mother and batch cultures have been propagated successfully in reconstituted skim milk for more than 2 years. In order to assure a milk satisfactory for growth and activity of starter cultures, it was found necessary to test each barrel of nonfat dry milk powder. The source of the water used in reconstituting the skim milk significantly influenced the activity of the cultures. The use of distilled water consistently yielded a satisfactory milk. Certain city and plant water supplies consistently yielded milks that failed to provide active cultures.

M18 Influence of the Per Cent Oxygen in the Headspace Gas of Container on the Quality of Dry Whole Milk during Storage for One Year.

G. H. WILSTER, Oregon State College.

This report covers one phase of a research project that involved the manufacture, storage, and rehydration of dry whole milk. The method of manufacture consisted of: (a) heating the standardized milk, which previously had been clarified centrifugally, in a stainless steel pasteurizer for about 45 to 50 minutes to a temperature to 170° F. and holding at this temperature for 30 minutes; (b) cooling the milk to 145° F.; (c) condensing to 3:1 concentration in a stainless steel vacuum pan 3 feet in diameter; (d) homogenizing at 1,500 lb. per square inch, first stage, and 500 lb. per square inch, second stage, at the temperature as obtained from the vacuum pan; (e) cooling on a stainless steel surface cooler to about 35° F.; (f) preheating the condensed milk in a 10-gallon capacity stainless steel tank; and, finally, (g) spray drying the condensed milk in a 6′ × 17′ × 9′ chamber.

When packing, 155 g. dry milk were weighed into size no. 1 tin containers. The cans then were sealed. A total of 71 packs, usually 24 cans to each pack, was made. When evacuating and gassing, the punctured cans were placed in an evacuation chamber and evacuated and gassed as desired. Immediately after removing the cans from the chamber, they were soldered quickly. The samples of dry milk for all packs were stored for 1 year at a temperature of 80 to 90° F. The flavor of the reconstituted milk, per cent oxygen in the headspace gas of container, per cent carbon dioxide in headspace gas of container, solubility index, and ascorbic acid content of the freshly reconstituted milk were determined at monthly intervals. The data show that as the per cent oxygen in the headspace of the container was decreased, the keeping quality of the dry milk improved. The average score of the milk packed with less than 1 per cent oxygen at equilibrium was 1.44 higher at the end of the storage period than that of the milk packed with 3.04 to 3.50 per cent oxygen. The final score of the milk in the low-oxygen group, at the end of the 12-month storage period, was 18.09 and that of the high-oxygen milk was 16.97. This was based on a maximum score of 25 for flavor.

The influence of size of the dry milk particles on the keeping quality was studied with eight batches of powder. The average size of the milk particles ranged from 8.15 to 19.29 μ in diameter. When the 32 packs of powder were grouped in accordance with the amount of oxygen in the headspace of the container at equilibrium and the decreases in the flavor score were averaged, the coarser-grained powder was found to possess better keeping quality than the fine-grained powder.

M19 A Comparison of the Yields of Cheddar Cheese Obtained from Raw, Holder Pasteurized and High Short-Time Pasteurized Milk. O. R. Irvine, L. R. Bryant, D. C. Hill, and W. H. Sproule, Ontario Agricultural College.

In connection with some wartime research on the nutritional value of Cheddar cheese, an opportunity was afforded to secure data on yields of cheese from milks receiving three types of treatment. The results of nine comparative trials are reported; in each trial approximately 1,800 lb. of carefully-mixed milk was divided into three lots. The first lot was made into raw milk cheese, the second was pasteurized to 143° F. for 30 minutes and then made into cheese, and the third lot was pasteurized at 161° F. for 16 seconds before manufacture.

Yields of the 1-day-old cheese from these vats were calculated in terms of 35 per cent moisture in order to eliminate this source of variation in the results. On this basis, the mean for the raw milk vats was 9.84 lb. of cheese per 100 lb. of milk, while the value for the holder pasteurized milk was 10.02 and for the high short-time pasteurized milk 10.01. Calculations based on determinations of the fat content of the milk and cheese reveal that 88.68 per cent of this constituent was retained in the case of the raw milk cheese, while the mean rates of butterfat retention were 90.15 and 91.13 per cent, respectively, for the holder and high short-time pasteurized milk. Fat determinations made on the wheys removed up to the time cheddaring of the curds was begun gave means which were approximately equal in the case of the raw and holder pasteurized milk cheese, but which were markedly lower in the case of the high short-time pasteurized milk cheese.

The data from which these values were derived were subject to statistical treatment by the analysis of variance method. No evidence exists that the yields of cheese per 100 lb. of milk are influenced by the different treatments which the milk received. However, the treatments did produce significant differences in the efficiency with which the butterfat was retained in the cheese as well as the rate at which butterfat was lost in the whey.

M20 A Study of the Volatile Acidity in Milk. P. G. Miller, P. L. Zimmerman, and E. B. Oberg, Carnation Company, Milwaukee, Wisconsin.

Since the heat treatment of milk has been shown to increase volatile acidity, a study of the volatile acids in processed milk should aid in evaluating different sterilizing treatments. The best method for obtaining volatile acids from milk without producing more acids is subject to question. Steam distillation has been reported to produce no formic acid; however, it does release volatile sulfide(s). On the other hand, vacuum distillation does not appear to release volatile sulfides and thus may be expected to be less destructive of the milk constituents.

Steam distillation and vacuum (at 24 mm. Hg. and constant volume) distillation procedures were compared on the basis of rate of distillation of formic acid and of *n*-butyric acid, the recovery of added formic acid, total volatile acidity in the distillates, and other factors. Some vacuum distillation results are presented showing the relation of sterilization treatment of evaporated milk to development of total volatile acidity, formic acid, and color.

The rate of distillation of formic acid was slightly greater with steam than with vacuum. By either method approximately 30 per cent of the formic acid was distilled when the volume of the distillate equaled the volume of the distilland. The rate of distillation of *n*-butyric acid definitely was greater with steam than with vacuum. With steam distillation the recovery was about 87 per cent and with vacuum distillation about 53 per cent, when the volume of the distillate equaled the volume of the distilland. Steam and vacuum distillations gave about the same recovery for added formic acid (gravimetric method).

Steam distillation of milk gave higher values for total volatile acidity than did vacuum distillation. The higher values with steam distillation appeared to be due to the evolution of CO_2 during the steam distillation and not to the more rapid distillation of acids such as butyric.

Steam distillation of an aqueous tributyrin suspension resulted in the hydrolysis of about 3.5 per cent of the triglyceride, whereas vacuum distillation showed no evidence of triglyceride hydrolysis. The total volatile acidity, formic acid, and color of evaporated milk varied directly with the degree of heat treatment.

M21 Some Unique Properties of Lactose as a Dietary Carbohydrate.

LLOYD K. RIGGS AND ANNABEL BEATY, Kraft Foods Company, Chicago, Illinois.

Young rats weighing 50 to 60 g. were fed *ad libitum* purified diets containing 5 per cent fat in which USP lactose was substituted for cornstarch at levels of 5, 10, 15, 20, 25, 30, 40, and 50 per cent of the total ration. Feed consumption was recorded and the animals were weighed and observed for symptoms of lactose feeding three times each week for 12 weeks. Up to and including 30 per cent lactose, these rations equaled the basal ration (no lactose) in growth and food utilization. At the 40 and 50 per cent levels growth was less, and at 50 per cent food utilization was less efficient than on the basal ration.

Symptoms of lactose feeding, diarrhea and/or "pot-bellies", were observed first at the 20 per cent level and became progressively more severe and more persistent as the percentage of lactose in the ration was increased. The capacity of the rats to utilize lactose without suffering from diarrhea was increased through repeated daily intake of rations containing lactose.

By feeding rations containing a mixture of equal parts glucose and galactose at levels equivalent to 15, 30, and 50 per cent lactose, the lactose linkage was found responsible for the symptoms observed on rations containing higher percentages of lactose. At levels of 30 and 50 per cent, growth was less and food utilization more efficient on the diets containing lactose than on the rations containing equivalent amounts of the mixture of the two simple sugars.

M22 The Relationship between the Oxidation of Ascorbic Acid and the Development of the Oxidized Flavor in Milk. George R. Greenbank and Philip A. Wright, Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

In recent publications attempts have been made to revive the enzyme theory to explain the development of the oxidized flavor. A study has been made to determine whether the reaction which accompanies the development of an oxidized flavor in milk resembles an enzymatic or a chemical oxidation. The oxidation of ascorbic acid, a normal constituent of milk, has been considered by others as a link in the postulated oxidation of the lipid which causes the off flavor. Data are presented to show that the oxidation of ascorbic acid parallels the postulated oxidation of the lipid but is not necessarily a link in the oxidation chain and that the addition or destruction of ascorbic acid promotes changes which may be expected when reducing agents are added to a milk or removed from it. Many seemingly abnormal reactions observed by others upon the addition of ascorbic acid, copper, and hydrogen peroxide to a milk are clarified by the use of a simple equation to illustrate the chemical changes which occur during the development of the flavor.

M23 The Use of Carotene for Coloring Butter. G. A. Richardson and M. Louisa Long, University of California.

Color determinations on the fat from 132 butters manufactured in California and collected in connection with a national survey of the vitamin A activity of butter revealed a surprising lack of uniformity of coloring. The variation in carotene content was not compensated for, adequately, by added color.

The intensity of color in terms of optical density was determined on a 10 per cent (w/v) solution of the butter in gasoline, using a 1.6 cm. cell, a wave length of 440 mμ, a wave band of 35 mμ, 20° C., with a Model 11 Coleman spectrophotometer. It was found that 7.6 per cent of the samples fell in the optical density range of 0.15–0.20, 16.7 in the range 0.21–0.25, 28 within 0.26–0.30, 31.1 within 0.31–0.35, 13.6 within 0.36–0.40, and 3 per cent within 0.41–0.45. If carotene were the only color present, these ranges represent, respectively, carotene values of approximately 3,700–5,200, 5,500–6,700, 7,000–8,200, 8,500–9,600, 9,800–10,800, and 11,000–12,000 I.U. per lb. of butter.

Carotene is known to be prooxygenic in refined oils, especially at elevated temperatures. It was found to be moderately prooxygenic in butter oil prepared so as to be low in phospholipid, α-tocopherol, and other antioxidants. With milk fat as usually prepared in the laboratory, added carotene is not definitely prooxygenic at room temperature in the absence of direct sunlight. Added α-tocopherol or lecithin imparts some protection but is not as effective as nordihydroguaiaretic acid (NDGA) or quercetin. Carotene dissolved in the fraction of milk fat liquid at room temperature (approximately 68° F.) is more rapidly destroyed than when dissolved in either the complete fat or in the fraction that has crystallized out at or above room temperature.

In the initial experiments, a commercial carotene concentrate (from carrots) in Wesson oil was used as added color for laboratory churnings. The color characteristics were good, but flavor scores, especially after storage, were unfavorable. Refinements in the methods of extraction and isolation of carotene from carrots have made available carotene concentrates of little or no flavor or odor, and of potencies ranging from 5,000 to 8,800 I.U. per g. The stability of these concentrates is quite high. More than 50 per cent of the carotene remained after exposure to laboratory light and temperature conditions for 40 days. Quercetin and NDGA decreased the rate of destruction.

Several laboratory churnings were made from the same lot of cream, with and without added copper (2 p.p.m.) and with and without added carotene. Samples were stored at 18 and at 60° F. At the end of a 10-month storage period at 18° F. the peroxide values of all samples were fairly low (av. 1.56 me/kg., without copper; 2.19 me/kg., with copper). Added carotene, at this temperature, neither promoted nor inhibited peroxide accumulation.

At the end of 5 months of storage at 60° F., the peroxide values of the controls without added copper were less than 2.5. Added carotene was definitely prooxygenic for those without copper but appeared antioxygenic for the samples containing added copper. The color values of all samples not containing copper remained practically unchanged during the 5 months of storage.

Butters manufactured under commercial conditions and colored to different degrees with one of three commercial carotene concentrates are undergoing storage trials.

A simple method is described by which the tester may determine the color score of the fat to be churned and indicate to the buttermaker the amount of color to be added per 100 lb. of fat to give a predetermined shade to the butter.

M24 Graphic Procedure for Determining Quantitative Results of Volumetric Analysis. WAYNE I. TRETSVEN, Advisory Service, Chicago, Illinois.

A graphic procedure is described for rapidly and accurately determining the results of volumetric analysis. The tedious tasks of preparing solutions to a definite concentration (normality) or their standardization to normality factors, and all the calculations involved, are eliminated by substituting a simple graph. It is particularly useful when two or more solutions are required and is especially advantageous where one or both solutions are of such nature as to require frequent restandardization. The procedure has been adapted to various analyses and is explained in terms of a specific example. Since the method was developed and used extensively in the laboratory for determining the salt content of cheese, it is illustrated in terms of this application.

, The principles of the procedure depend on establishing a line or lines portraying the actual titration values of the reagents. Only two points (titrations) are necessary. The points established represent an actual analysis and are based on a 100 per cent recovery. The construction of the graph is simple. If the scales of the graph are chosen suitably, *e.g.*, 1.0 division per 0.1 ml. solution or per 0.1 mg., the results will be as accurate as the burettes can be read. In actual use, no knowledge of the normalities of the solutions or any calculations are necessary.

M25 Some Chemical Reactions Involved in the Production of the Sunlight Flavor in Milk.

D. G. KEENEY AND D. V. JOSEPHSON, Department of Dairy Technology, Ohio State University.

The sunlight flavor precursors are known to originate in the milk serum, although the reactive compounds involved in the photochemical changes have yet to be identified.

In order to ascertain the type of reaction involved in the production of the sunlight flavor, attempts were made to synthesize a similar flavor. By the addition of approximately 50 p.p.m. of formaldehyde before heating the milk to 75° C., a flavor was developed which was indistinguishable from that produced by exposing the same untreated milk to sunlight. Heat treatments below 75° C. resulted in no flavor change in formaldehyde-treated milks, while heating to 80–90° C. served to intensify the sunlight-type flavor development. The critical temperature for sunlight flavor development under these conditions coincides with that for the liberation of sulfhydryl compounds in normal heated milk. This would suggest that sulfhydryl compounds and aldehyde groupings are essential to the mechanism of the sunlight flavor development, possibly the formation of a mercaptal type compound. Substantiating evidence was furnished by the fact that in heating formaldehyde-treated milks the cooked flavor never developed, and free sulfhydryl compounds as indicated by the nitroprusside test were greatly diminished or absent.

The oxidation-reduction range and poise seem to play an important rôle in both the natural and synthetic development of the sunlight flavor.

When casein was removed from skim milk by rennet coagulation and the albumin subsequently eliminated by boiling and supercentrifuging at 35,000 r.p.m. for 0.5 hour, the resultant clear liquid upon exposure to sunlight developed the flavor to such an extent that it was still discernible upon dilution to ten times its volume with the original skim milk. The development of the flavor in this fraction always was accompanied by a definite increase in saltiness.

Upon dialysis of skim milk for 36 hours and subsequent exposure of the fraction to sunlight, no detectable sunlight flavor developed. When skim milk first was exposed and then dialyzed, the sunlight flavor of the nondialyzable fraction showed a decrease far greater than could be attributed to dilution. In no case was a detectable sunlight flavor found in even the concentrated phase of the diffusate. This would suggest that the flavor compound either exists in a complex, all substituents of which are essential for the flavor, or that the flavor is sensitive to changes in the oxidation-reduction potential and had been destroyed by the dialytic action.

M26 A New Sensitive Method for Determining the Concentration of Quaternary Ammonium Germicide Solutions.

W. J. Harper and P. R. Elliker, Department of Dairy Husbandry, Purdue University, and W. K. Moseley, Moseley Laboratories, Inc., Indianapolis, Indiana.

An accurate, rapid, simple test for determining the concentration of quaternary ammonium germicides has been developed. The method is based on the formation of a red precipitate on addition of 1 ml. of quaternary ammonium solution to an equal volume of eosin-yellowish dye solution (0.01 per cent eosin-yellowish dissolved in acetone and the acetone solution diluted 1:10 in tetrachlorethane) in the presence of 0.1 ml. of pH 3.5 citric acid-NaOH buffer, and the subsequent titration to a colorless compound with a 0.01 per cent active anionic surface-active reagent. A standard curve may be established for a specific quaternary by using a pure compound of known concentration.

The method has been found satisfactory for determining the concentration of a number of different dilute quaternary ammonium solutions. It may be used either as a quantitative laboratory method or as a rapid field kit test. It also may be used to detect the quantity of quaternary ammonium germicides added to raw milk. A modification for estimating the concentration of quaternaries by the use of paper test strips has been developed.

M27 Ionic Exchangers in the Dairy Industry.

O. F. Garrett, M and R Dietetic Laboratories, Inc., Columbus, Ohio.

Ionic exchangers have been known and used in the dairy industry of the United States since the early 1930's when Lyman obtained a patent on a process for treating milk products. Lyman's process employs the base exchange principle, which is a type of ionic exchange, and is broad in its applications covering the treatment of milk, skim milk, whey, and the preparation of milk for evaporation, cheesemaking, etc. The development of new types of ionic exchanger materials in the last few years has stimulated experimental work looking toward the application of these materials to the processing of dairy products.

Experimental investigation of the application of ionic exchangers to the manufacture of various products from milk has been continuous in our laboratories beginning in 1932. The application of the base exchange principle to the preparation of soft curd milk is well known in the industry. In addition we have found that ionic exchanger materials may be applied to ice cream making, powdered whole milk, condensed milks, stabilized condensed and dried creams, lactose manufacture, and other specialized products.

M28 A Microscopic Study of Ice Cream Texture. W. S. Arbuckle, Animal Industry Department, Dairy Manufacturing Section, North Carolina State College.

The microscope is useful in determining certain body and texture characteristics of ice cream which are not readily detected organoleptically. The method used for routine examination includes the preparation of a thin section of ice cream, imbedding the section in an oil with a refractive index of 1.420 and examining with a microscope at a magnification of 100 times. Sectioning and examining are best done at a temperature of − 15° F. or lower.

Lithographed photomicrographs are included in this paper to illustrate the effect of type of freezer, heat shock, and other factors on the structure of ice cream.

M29 The Place of the Private Quality Control Laboratory in the Dairy Industry. M. A. Collins, Collins Dairy and Food Laboratories, Dryden, N. Y.

Factors to be considered in the establishment and operation of a private laboratory serving the dairy industry are discussed. Tests on the various dairy products, farm inspection, plant inspection, efficiency of plant operations, and personnel training are services which a laboratory of this type may offer.

M30 Newly Developed Cleaning Aids for the Dairy Industry. John R. Perry, Sealtest, Inc., New York, N. Y.

Devices recently developed by the author are designed to make possible better, more efficient, and more economical cleaning of dairy equipment and to make the cleaning job a desirable one instead of the distasteful one it so generally has been. Among the devices described are the shut-off valve for the end of the rinse hose, the sanitary fittings cleaner, the sanitary pipe cleaner, the separator disc cleaner, solution fed brushes, special brushes, and the power-driven solution fed brush.

M31 Some Observations on the Chemistry of Heat-induced Flavors in Milk. Stuart Patton and Donald V. Josephson, Ohio State University.

The heat-induced flavor changes occurring in heated skim milk were studied, using a group of five taste observers. The most important and pronounced changes resulting from the heat treatment were found to be the development of "cooked" (olfactory) and "rough" (tactual) flavors. These same flavors also were manifest to approximately the same degree in heated rennet whey.

In order to determine the importance of serum proteins in these flavor changes, heated (205° F.—flash) rennet whey was supercentrifuged at 35,000 r.p.m. Such treatment produced significant reductions in "cooked" as well as "rough" tactual flavor responses. Parallel diminution (in certain cases complete) in the nitroprusside test of the whey occurred as the result of the removal of denatured serum proteins. The primary importance of serum proteins in heat-induced flavor changes was demonstrated by these studies. Attempts to remove serum proteins from heated skim milk by supercentrifugation were unsuccessful, as indicated by the nitroprusside test and flavor responses of the observers to the skim milk following centrifuging treatment. This phenomenon may be attributed to the physical and/or chemical adsorption of serum proteins on the casein micelles in heated milk.

In studying the behavior of sulfhydryl groups in heated milk, it was found that the serum proteins (sulfhydryl positive) dispose themselves in the rennet curd of the milk rather than in the whey. Such a phenomenon may explain, in part, the soft curd characteristics of highly heated milk. The close physico-chemical relationship existing between serum proteins and casein in heated milk also is demonstrated.

When samples of skim milk were heated for prolonged periods at 205° F., the quantity of sulfhydryl groups, as determined by the nitroprusside test, was found to diminish substantially. However, in the absence of casein (rennet whey) and lactose (dialyzed skim milk), such a diminution did not occur. These observations may serve to elucidate the nature of carmelized flavor development in heated milk.

M32 The Utilization of the Mineral-Ion Exchange Principle in Stabilizing Evaporated Milk. D. V. JOSEPHSON AND C. B. REEVES, Department of Dairy Technology, Ohio State University.

The proteins in evaporated milk usually are somewhat unstable to the heat of sterilization. This condition has been attributed primarily to an excess of calcium ions and, possibly, of magnesium ions and is corrected in commercial manufacture by the use of certain salts, usually disodium phosphate.

The present study was undertaken to investigate the possibility of using some ion exchange method to remove the excess calcium and magnesium ions from milk and render the concentrated product stable to 240° F. for 15 minutes. A cation exchanger (Amberlite IR–100) which exhibits a high degree of specificity for polyvalent cations was used for treating whole milks and skim milks. When this resinous type material was used in the sodium cycle, it effected an efficient removal of calcium (60 per cent) and magnesium from milk.

The mineral-ion exchange treated milks were added as fluids, concentrates, and powders to the bulk lots of regular fluid milk before forewarming or to the untreated concentrates before sterilization. Under average conditions where evaporated milk required 2 to 6 ounces of disodium phosphate per 1,000 lb. of concentrate to produce stability, the same correction was effected by treating from 0.5 to 2.0 per cent of the original milk (or equivalent) by the mineral-ion exchange process. The addition of the mineral-ion exchange treated milk at the hot well before forewarming produced greater efficiency of stabilization than addition later.

The mineral-ion exchange treated milk can be used as a fluid, concentrate, or powder with equal effectiveness. Both whole and skimmed milks can be treated by the process, but skim milk is more practical from the commercial point of view. Changes in the calcium, phosphorus, and ash content of evaporated milk as a result of mineral-ion exchange treatment are so insignificant as to be within the limits of error for the analytical techniques used. The mineral-ion exchange process as employed in these experiments with evaporated milk appears to be a practical procedure for the control of heat coagulation of evaporated milk.

M33 The Manufacture of High-Solids Evaporated Milk. MARK KEENEY AND DONALD V. JOSEPHSON, Department of Dairy Technology, Ohio State University.

This study was undertaken to investigate the possibility of manufacturing a commercially-acceptable evaporated milk containing an appreciably greater total solids content than the present standard product of 26 per cent total solids. Attention was focused on the manufacture of evaporated milk at levels of 32, 34, 36, 38, 39, and 40 per cent total solids.

The intensification of the physical and chemical changes in milk caused by the heat of sterilization at high solids concentration has handicapped the development of such a product. Prohibitive amounts of disodium phosphate are necessary during most seasons of the year to stabilize evaporated milk containing 34 to 40 per cent solids. In this study the mineral-ion exchange principle was used and compared with the procedure of stabilization by disodium phosphate.

During January, February, and March the 40 per cent solids evaporated milk could be stabilized against heat coagulation by the addition of 8 to 11 per cent (on a solids basis) of mineral-ion exchange powder (from which 60 per cent of the calcium had been removed) at the hot well. The same milks, untreated, required from 70 to 85 ounces of disodium phosphate per 1,000 lb. of 40 per cent solids milk to prevent coagulation.

As the solids content was increased, the degree of browning was intensified. The products reconstituted to give 13 per cent solids exhibited lower pH as the solids concentration of the sterilized product increased. A normal milk which exhibited a pH of 6.55 before sterilization usually had a pH of about 6.35 after sterilization at a normal concentration. The pH of sterilized high solids concentrates decreased as solids increased, so that a value of 6.20 or lower was common with a 40 per cent solids product (reconstituted).

Sterilization causes a considerable change in the optically active lactose content of evaporated milk. The per cent destruction of optically active lactose ranged from 9 to 15 per cent. The per cent solids at sterilization seemed to have little or no effect upon the per cent destruction.

All of the batches containing 32 per cent or more solids left a noticeable mineral deposit in the cans within a few days after sterilization. This was true in both the mineral-ion exchange treated batches and the products stabilized with disodium phosphate.

M34 Condensing Whole Milk for Ice Cream Mix with the Vacreator.

G. H. Wilster and K. C. Lu, Oregon State College.

The process of condensing whole milk with a "baby" size Vacreator was studied. The whole milk first was placed in a storage vat and then pumped either through a flash preheater or a tubular preheater, heated to 200 ± 5° F., and evaporated under 24 and 28 inches of partial vacuum, consecutively, in the Vacreator. The partially condensed whole milk was returned to the storage vat as it emerged from the machine. By continuous circulation through the Vacreator, the milk was condensed to the desired ratio of concentration of about 2.1 : 1 for use in ice cream mix.

Increasing the rate of inflow of milk to the Vacreator up to its maximum intake capacity resulted in a higher water-evaporating efficiency. An increase in the rate of inflow of the milk from 85 to 105 lb. per minute increased the water-removal capacity of the machine from an average of 6.39 to 8.04 lb. per minute (383.4 to 482.4 lb. per hour).

The average bacterial count of the milk in the storage vat dropped from 85,600 per ml. before condensing to 1,500 per ml. after 30 minutes of condensing. The average bacterial count of the condensed milk when leaving the Vacreator at the conclusion of the process was 100 per ml.; the count of the finished condensed product in the storage vat was about 200 per ml.

The Vacreator-condensed whole milk stored at 80° F. was satisfactory for about 12 hours, while at 44° F. the milk kept satisfactorily for about 1 week. When 5 per cent of sucrose was added to the condensed milk and the milk stored in 1 pint cardboard containers at 0 to 10° F., the milk kept satisfactorily for 34 days. The reconstituted milk had a fine flavor and showed no protein destabilization.

One of the problems in condensing milk with a Vacreator for an ice cream plant is the determination of the total solids content of the condensed product. Equations that show the relationship between the total solids (Y), the Baumé reading (X), and the fat content (Z) for the condensed whole milk made from high testing and low testing milk and also for the condensed skim milk were formulated. The equation most valuable when milk of high test was used was $Y_E = 1.5642\,X + 1.5020\,Z - 0.3286$, with a multiple correlation coefficient of 0.9989 and a standard error of estimate of 0.2112. When milk of low test was used, the most valuable equation was $Y_E = 1.9012\,X - 0.0368$, with a correlation coefficient of 0.9999 and a standard error of 0.0151.

M35 A Study of the Use of Nordihydroguaiaretic Acid in the Storage of Frozen Sweet Cream. J. W. Stull, E. O. Herreid, and P. H. Tracy, Illinois Agricultural Experiment Station.

A study has been made of the effectiveness of the antioxidant nordihydroguaiaretic acid (N.D.G.A.) in retarding or preventing the development of the oxidized flavor in unsweetened frozen cream. Four lots of cream were prepared. These consisted of high and low quality cream pasteurized both at 150° F. for 30 minutes and 170° F. for 15 minutes. Each lot was divided into two series, one with the copper and N.D.G.A. added before pasteurization and the other with the copper and antioxidant added after cooling to 45° F. The copper was added from an aqueous solution to make a concentration of 0.5 p.p.m. based on the total weight of the cream. The N.D.G.A. was added from a glycerol solution and a water suspension to make concentrations of 0.00125, 0.0025, and 0.005 per cent of the fat content of the cream. The cream was stored in tinned cans at a temperature varying from – 5° to – 20° F. When taken out of storage, the cream was thawed and judged for flavor. The oxidation-reduction potential (Eh) of the cream was determined at this time. In order to accelerate further the development of the oxidized flavor, the cream was held at 40° F. for an additional week and then judged again.

In storing the cream for 7 to 9 months at subzero temperatures, it was found that the presence of from 0.00125 to 0.005 per cent N.D.G.A. would retard the development of the oxidized flavor. The glycerol and the water solutions were equally effective. The Eh of the cream increased slightly during storage. However, there was no significant difference in the Eh values of those samples which had the oxidized flavor and of those which did not have this off flavor at the end of comparable storage periods. These results indicate that Eh values for storage cream have no particular significance in measuring the development of the oxidized flavor.

In every case the copper which was added to the cream after it had been cooled to 45° F. induced the oxidized flavor more rapidly than the copper which was added to the cream prior to pasteurization. Pasteurization at 170° F. for 15 minutes protected the cream against the development of the oxidized flavor to a greater extent than pasteurization at 150° F. for 30 minutes.

The intensity of the oxidized flavor increased in the control samples which were held at 40° F. for 1 week whereas this did not occur in the samples which contained N.D.G.A. Control samples not oxidized when they first were taken out of storage often developed oxidized flavor during storage at 40° F.

PRODUCTION SECTION

P1 The Influence of Streptomycin upon the Livability and Bacterial Content of Bull Semen. J. O. Almquist, W. T. S. Thorp, and P. J. Glantz, Pennsylvania Agricultural Experiment Station.

Investigations on the use of penicillin in diluted bovine semen led to subsequent studies with streptomycin. Preliminary studies indicated that levels of 2,500 to 10,000 μg. of streptomycin per ml. of diluted semen markedly reduced the ability of spermatozoa to maintain motility during storage. Thus an experiment was designed in which streptomycin was added to 10 samples of bull semen diluted 1 to 24 with yolk-citrate at the rate of 0, 100, 250, 500, 750, 1,000, 1,250, 1,500, and 2,000 μg. per ml. of diluted semen. The samples were stored at 4.5° C. (40° F.) and the percentages of motile spermatozoa were estimated every 2 days for 20 days. At 0, 8, and 16 days' storage, subsamples were taken for bacterial counts and streptomycin assays.

Analysis of variance involving 900 motility estimations showed no significant differences in spermatozoan livability at levels of 0, 100, 250, 500, 750, and 1,000 μg. However, the three highest levels tested (1,250, 1,500, and 2,000 μg. per ml. of diluted semen) caused a highly significant decline in livability. Based on a highly significant linear regression, the mean percentage of motile spermatozoa during 20 days' storage decreased by 0.5 per cent for each additional 250 μg. of streptomycin.

Plate counts were made on nine samples of diluted semen stored for 0, 8, and 16 days. Veal infusion agar containing 4 per cent ox blood was used for total bacterial counts, and desoxycholate agar was used for enumeration of bacteria belonging to the coliform group. The blood agar plates were incubated for 48 hours at 37° C., while the desoxycholate agar plates were held for 24 hours at 37° C.

Levels of streptomycin above 100 μg. per ml. of diluted semen inhibited the growth of bacteria. Freshly diluted semen without streptomycin contained an average of 5,000 bacteria per cc., as compared to an average of 120 bacteria per cc. for samples with added streptomycin. After 8 days' storage at 4.5° C., the tubes of untreated semen averaged 82,000 bacteria per cc. while those containing streptomycin averaged 2,000 bacteria per cc. of diluted semen. After 16 days' storage the average bacterial count for diluted semen without the antibiotic was 131 million per cc. and 137,000 per cc. for diluted semen containing streptomycin.

Very few typical coliform bacteria were present on the desoxycholate agar plates. Only one sample had countable colonies and these were present only in the undiluted semen and the tube of freshly diluted semen which received no streptomycin. The number of coliform bacteria increased in these two tubes during the 16-day storage period.

Streptomycin was stable for 16 days in diluted semen stored at 4.5° C., as determined by assays with the standard cylinder plate method using *Bacillus subtilis* as the test organism. Studies are now in progress using streptomycin in combination with penicillin, since it appears that streptomycin might aid in controlling certain penicillin-resistant organisms sometimes found in semen.

P2 **Some Effects of Adding Thyroxine to Bull Semen.** A. B. SCHULTZE AND H. P. DAVIS, University of Nebraska.

d,l-thyroxine was added in 0.3, 0.7, 1.0, and 4.0 μg. quantities to 10 ml. of diluted bull semen. The effect of these additions of thyroxine on the oxygen consumption of the sperm was determined by a Barcroft-Warburg respirometer.

An average of ten determinations showed that the 0.3 μg. level increased the oxygen consumption 5 per cent above the control. The 0.7 μg. level resulted in an average increase of 9 per cent in oxygen consumption above the controls (24 determinations). Four of the 24 samples were retarded in oxygen consumption by the addition of thyroxine. The 1.0 μg. level increased oxygen consumption 4.6 per cent above the controls (27 determinations). Six of the 27 samples showed retardation of oxygen consumption from the addition of thyroxine. The 4.0 μg. level increased oxygen consumption 4 per cent (13 determinations). Six of the 13 samples were retarded in oxygen consumption by the addition of thyroxine. The addition of 80 μg. to 10 ml. of diluted semen resulted in inhibition of oxygen consumption to 92 per cent of the control level for 4 determinations. The majority of the determinations were made the day following the collection and treatment of the semen. The thyroxine was added 1–2 hours after collection and dilution of the semen.

Seventy semen samples treated with 1 μg. of d,l-thyroxine per 10 ml. diluted semen and used in artificial breeding associations showed a conception rate of 61.4 per cent, based on a 5 months' non-return period (1,623 services). Untreated semen samples from the same bulls during the same period (2,954 services) showed a 56.7 per cent conception rate.

P3 Total Digestible Nutrients and Protein Levels for Dairy Bulls Used in Artificial Insemination.

CECIL BRANTON, R. W. BRATTON, AND G. W. SALISBURY, Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry, Cornell University.

The increasing number and size of bull studs supplying semen for artificial insemination has focused attention on the inadequacy of quantitative information relating the feeding of different levels of total digestible nutrients and total protein to semen production, fertility, and body weight changes of bulls.

From this general situation the problem was reduced to one of relating controlled levels of T.D.N. and protein intake to the changes in measurable characteristics of semen quantity and quality, relative fertility, and changes in body weights.

Using nine Holstein and nine Guernsey bulls, T.D.N. levels of 100, 120, and 140 per cent of Morrison's dry dairy cow maintenance requirements were studied by means of a "double change-over" experiment involving three 90-day periods. The T.D.N. requirements were based on the initial body weights of the bulls. A continuous-type protein experiment involving the feeding of concentrate mixtures containing 12, 16, and 20 per cent total protein was superimposed on the T.D.N. experiment. Forty per cent of the T.D.N. requirement was furnished by the concentrates and the remaining 60 per cent from mixed hay containing approximately 10 per cent of legumes.

Neither the quantity nor the quality of the semen was related to the level of T.D.N. fed the bulls. Significantly higher numbers of spermatozoa per mm.^{3}, lower volume per ejaculate, motility, and fewer total spermatozoa per ejaculate were produced by the bulls fed the concentrate containing 20 per cent total protein as compared to those fed the other mixtures. These differences are not considered at present to be of practical importance.

The fertility of the bulls as judged by 5-months' non-returns to first services from the semen samples used for insemination did not vary significantly with either the T.D.N. or protein level fed.

All levels of T.D.N. fed resulted in significant changes in body weights. On the other hand, protein levels and the interaction of protein and T.D.N. did not result in significant changes in body weight. Based on the linear regression between initial body weight and the estimated daily T.D.N. required to maintain constant body weight, the following practical feeding standard for dairy bulls in active service tentatively is suggested: 1 lb. of hay plus 0.4 to 0.5 lb. concentrate mixture containing about 12 per cent total protein per 100 lb. of body weight.

P4 A Study of Factors Affecting the Length of Gestation in Dairy Cattle.[1] H. A. Herman and R. W. Spalding, Department of Dairy Husbandry, University of Missouri.

Factors affecting the length of the gestation period of dairy cattle in the Missouri Station herd for the period 1900–1945 were studied. The average length of gestation in 1,957 cases, all twins and abortions excluded, was found to be 279.4 ± 5.59 days. This study included 876 Jerseys, 962 Holsteins, and 119 Guernseys.

The average length of gestation for the Jerseys was 280.36 ± 5.80 days. Four hundred and twenty-nine Jersey males were carried an average of 280.90 ± 5.38 days and 447 Jersey females an average of 279.85 ± 4.25 days. The average length of gestation for the Holsteins was 278.11 ± 4.98 days. Four hundred and ninety-five females were carried an average of 277.51 ± 3.79 days and 467 males an average of 278.75 ± 5.94 days. The average length of gestation for the Guernseys was 284.00 ± 4.96 days. Sixty-five males were carried an average of 284.57 ± 4.97 days, a day longer, on the average, than females.

Twins (29 pairs) and triplets (1 set) comprised 1.5 per cent of the births in the Missouri Station herd. The average length of gestation for multiple births, omitting all abortions, was found to be 271.56 ±8.93 days.

The data indicate a slight increase in the gestation length for mature cows as compared to the gestation period of first- and second-calf heifers. Cows calving during the fall and winter months carried their calves an average of 1 to 3 days longer than those cows, which calved during the spring and summer months. Animals positive to the agglutination test in 1:100 dilutions (57 cases) were found to have an incidence of premature births of 35 per cent, and only 65 per cent of the offspring was carried above 265 days. Animals reacting in dilutions of 1:25 and 1:50 were found to carry 91 per cent of their calves more than 265 days. The incidence of premature births in these two groups was 8.47 per cent and 9.09 per cent, respectively. The distribution of abortions in terms of gestation length indicates that 87 per cent of these occur after 5 months of pregnancy. The mean number of days pregnant at which abortions occurred was found to be 193.90 ± 52.19 for Holsteins (51 cases), 209.12 ± 43.15 for Jerseys (81 cases), and 229.25 ± 63.81 for Guernseys (4 cases).

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[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1051.

P5 The Relationship of Semen Production, Age, Plasma Calcium, and Plasma Phosphorus to the Plasma Phosphatases of Dairy Bulls.'

J. T. Reid, G. M. Ward, R. L. Salsbury, and C. E. Shuart, Rutgers University.

Although few investigations have been made of the plasma phosphatases in dairy cattle, numerous accounts are found in which the alkaline plasma phosphatase is related to bone metabolism in other species. A study was made of the relationship of the plasma phosphatases to semen production, plasma calcium and phosphorus, and age of dairy bulls from which semen was being obtained at various rates.

In 144 analyses made of the plasma of 33 bulls, the average alkaline phosphatase content was 8.98 units per 100 ml. (range 1.99–21.03) and the average acid phosphatase was 2.09 units per 100 ml. (range 0.35–6.63).

The level of the alkaline plasma phosphatase varied inversely with the number of spermatozoa produced. The mean phosphatase units found in the plasma of bulls producing within the following ranges of spermatozoa concentration $\times 10^8$ daily were: 0.00, 15.96; 2.00–4.50, 11.84; 4.51–7.00, 11.28; 7.01–9.50, 9.61; 9.51–12.00, 6.99; 12.01–14.50, 6.90; and 14.51–16.00, 4.66.

An average plasma level of 4.93 alkaline phosphatase units per 100 ml. was found for 10 bulls yielding an average of one ejaculate per 2.5–3.5 days. When the same group of bulls was rested for a period of 52 days, the average enzyme concentration was 10.95, or an increase of 2.2 times. One bull from which no semen ever had been obtained showed an average phosphatase concentration of 18.95 units (range 18.04–19.75 in five monthly analyses). After nine samples of very dilute semen totaling 193 ml. had been collected by massage technique during a 16-day period, the enzyme level dropped abruptly to 5.24 units.

The coefficients of correlation between the level of alkaline plasma phosphatase and several factors involved in the rate of semen production were: number of ejaculates per day, –0.71; volume of semen per day, –0.83; and number of spermatozoa per day, –0.86. The daily averages were based on the number of ejaculates and semen produced during a 3-month period prior to plasma analysis.

No relationship existed between the plasma concentration of acid phosphatase and the frequency of semen collection, or quantity of semen produced. Also, no relationship was found between the concentration of the enzyme in the plasma and the plasma levels of calcium and inorganic phosphorus, age of bulls, or ration fed.

These data would indicate that the quantity of semen, more particularly the number of spermatozoa, produced by dairy bulls is the major factor determining the concentration of alkaline phosphatase in the blood plasma.

P6 Light Variation Associated with Conception Rate in Artificial Breeding. ERNEST MERCIER, Ministry of Agriculture, Quebec, Canada, AND G. W. SALISBURY, Department of Animal Husbandry, Cornell University.

Many studies conducted on the breeding records of cattle have shown that reproductive efficiency may be associated with climatic factors. Since seasonal fluctuations in fertility level of cattle occur at high latitudes where the summer temperature does not reach the point of hyperthermal rise, the authors have been interested in determining what other climatic factors were involved.

A study of the breeding records of three herds of cattle located 2° of latitude apart in eastern Canada revealed that the conception rate of these herds was significantly lower during winter and spring than during summer and fall.

The results of four similar studies, involving 150,000 breeding records of groups of cows and bulls of various ages bred artificially in New York State, paralleled those obtained in eastern Canada. In every study, winter was found to be the poorest breeding season of the year. Furthermore, the results showed that the fertility level of cattle of extreme age, either young or old, fluctuated more from season to season than that of mature cattle or cattle of intermediate age. In every case the differences between seasons within age-groups, as well as seasons for all age groups, were statistically significant at either the 5 or the 1 per cent level of probability.

In both locations the over-all fertility level of cattle was found to be highly correlated with the length of daylight, with a lag of 1 to 2 months before the maximum effects of day-length variations were reached. The results of a partial correlation study indicated that variations in outside temperature were of no detectable importance on conception rate of cattle in eastern Canada.

Seasonal variations in length of daylight are believed to influence markedly the fertility of cattle at high latitude.

P7 Studies of Oxidative Mechanisms in Bull Semen. J. T. REID, R. L. SALSBURY, G. M. WARD, AND G. I. LEONARDI, Rutgers University.

Numerous investigations have evaluated the ascorbic acid concentration of the semen of various species by virtue of this reductant's ability to reduce 2, 6-dichlorophenolindophenol under controlled conditions of pH and reaction rate. Some of these studies have indicated a relationship of dye-reduction by semen to the fertility state of bulls.

The first portion of this study involved an investigation of the relationship between various bull semen characteristics which are used in quality ratings and the capacity of semen to reduce potassium iodate and 2, 6-dichlorophenolindophenol. A volumetric method using potassium iodate and the commonly employed photelometric dye-reduction method were used in the analysis of more than 400 semen samples.

No great degree of relationship existed between the reducing capacity of semen and the various semen characteristics; however, the concentration of spermatozoa showed the highest degree of relationship to the reducing property.

A high degree of correlation (0.906 ± 0.008) existed between the amount of reduction measured by the potassium iodate and the dye-reduction methods and appeared to be the result of measuring the same reductant(s) by both procedures. This observation initiated the second portion of the study, which dealt with an attempt to designate the amount of reduction attributable to several substances (ascorbic acid and glutathione) known to be present in animal tissues. Although this study is not yet completed, it was found by various treatments that sulfhydryl substances may constitute from 7 to 81 per cent of the reduction in semen generally attributed to ascorbic acid as determined by the reduction of dichlorophenolindophenol dye. The dye-reduction method under the conditions commonly employed to measure ascorbic acid was capable also of determining glutathione in pure solution and of measuring glutathione added to semen.

P8 Collecting Genetic Data through Cooperating Dairymen. N. P. Ralston, S. W. Mead, and W. M. Regan, University of California.

A dairy cattle breeding experiment at the University of California has been in progress for 29 years. It is designed to study, through inbreeding, the inheritance of milk and fat production and related factors.

A loan bull project was initiated in 1926. Its purpose was to increase the number of females available for the proving of young inbred sons of University herd sires, in order to study more fully the genetic constitution of the University experimental herd and to aid in perpetuating the inbred lines. At present there are nine cooperating dairymen and a number of test herds which have been in the project for 15 or more years.

The cooperators, who are selected carefully, agree to: (a) maintain membership in a cow testing association; (b) practice an approved disease control program; (c) keep accurate records of breedings and calvings, sex of offspring, and other pertinent information relative to the experiment; and (d) retain and test, during their first lactation, all daughters of the loan bulls.

The close association between the University investigators and the dairymen has aided materially in the management of the herds in order to provide useful experimental data. Members of the University staff make semi-annual visits to each herd and transfer all data to complete herd books and other necessary forms to effectively direct the study. Bulls are loaned according to a planned program to yield the desired information.

The cooperating herds provide approximately 500 females per year. Of the 50 bulls loaned, 39 have been proved, supplying over 1,300 first-calf daughter and dam records. In addition, these herds have supplemented the data collected in the University herd on the inheritance of seven recessive defects, the effect of inbreeding on calf mortality, and other data in the process of being assembled.

P9 Variations in Type Ratings of Individual Ayrshire Cows. GEORGE HYATT, JR. AND W. J. TYLER, West Virginia University.

Type classification programs have been adopted by all the major dairy cattle breed associations. It seemed that the variation in the type of dairy cows throughout a year and from year to year needed to be known before a definite appraisal of type classification programs could be made. Therefore, a study of the variations in type ratings of individual cows classified several times a year was undertaken in 1942 in the Ayrshire herd (known as the Reymann Memorial Herd) at the West Virginia Agricultural Experiment Station. This herd represents approximately 25 years of a continuous, carefully controlled breeding project. All the females that had calved were rated for type approximately three times per year from May, 1942, to December, 1946, by an official inspector appointed by the Ayrshire Breeders' Association. The standard grades of Excellent, Very Good, Good Plus, and Fair were used.

Eighty animals were rated officially three times or more from May, 1942, to December, 1946. Seven animals were rated the same each time they were classified, 47 had a range of one grade, 23 a range of two grades, and three varied three grades.

The repeatabilities between ratings given a cow by the same inspector and between ratings given by different inspectors were computed after the data were analyzed by the analysis of variance. The average correlation coefficient between the ratings of each cow classified by inspector A in May, 1942, October, 1942, and May, 1943, was 0.74. For inspector B, whose ratings were made in October, 1943, and January, 1944, the correlation coefficient was 0.82; and for inspector C, who made his inspections in July, 1944, and May, 1946, it was 0.62. The repeatability of the ratings of 101 cows classified two or more times by different inspectors was 0.55.

The effect of age on variation of type ratings was studied using 65 females that were classified numerous times at 3 and 4 years of age, 30 classified several times at 4 and 5 years of age, and 27 rated several times at 3, 4, and 5 years of age. There is a small upward statistically significant change in type ratings as the animal matures.

The stage of lactation as a source of variation of type ratings was studied by analyzing the ratings of 63 animals classified 137 times between freshening and the fourth month of lactation, inclusive, 140 times between the fifth and the ninth month of lactation, inclusive, and 173 times between the tenth month of lactation and the next freshening period. The differences are small but do suggest that a little higher rating is obtained when animals are classified shortly before or after freshening rather than in the middle part of their lactation.

Most of the wide variations in type ratings of individual cows apparently can be attributed to the inability of the inspectors to appraise equally certain faults of a cow, especially defects in udder, feet, and legs. For example, 22 of the 26 cows that had a range of two or more grades changed at least two official grades in two consecutive classifications (an interval of 3 to 6 months). For 19 of these animals the main faults recorded were crooked legs, bad feet, or udders defective in either shape or attachment.

These results indicate that uniform cuts for the important defects in feet, legs, and udder should be adopted and adhered to as closely as possible by the various inspectors. An average of several type ratings of an individual cow seems a more accurate guide to her true type conformation than one official rating.

P10 The Heritability of Official Type Ratings and the Correlation between Type Ratings and Butterfat Production of Ayrshire Cows.

W. J. TYLER AND GEORGE HYATT, JR., West Virginia University.

The objectives of this study in dairy cattle breeding were to estimate the portion of the differences in official type ratings that is transmitted from parents to offspring and to compute the correlation between type rating and butterfat production of cows (records adjusted to a 305-day, mature equivalent, twice-a-day basis).

The classification ratings of 3,738 paternal half sisters and of 1,601 cows out of classified dams were used to estimate the heritability of single type ratings. From these data this figure was estimated to be 0.3, with 95 per cent fiducial limits of 0.19 and 0.41. These results suggest that selection for type through a type classification program should improve the type of future generations of dairy cattle.

The relationship within herds between classification rating and production of butterfat was computed from data on 5,177 cows. There was an average increase of 13 lb. of butterfat for each increase of one type grade. The correlation coefficient was 0.16 between the type rating and first butterfat record, 0.16 between type rating and the butterfat record begun nearest the date of classification, and 0.19 between type rating and the average of all the butterfat records of the cow. These coefficients were statistically significant, but their size indicates limited practical significance. This would indicate that selection within herds for better type only would not result in any substantial increase in butterfat production. Likewise, selection for increased production of butterfat alone would not improve the type of the animals to any great extent.

P11 Analysis of the Production Records of Crossbred Dairy Cattle.

R. A. Hilder and M. H. Fohrman, Bureau of Dairy Industry, U. S. Department of Agriculture.

A popular notion about crossbreeding is that the offspring resulting from interbreed crosses might show wide variation in butterfat production. This analysis is undertaken to show whether this variation exists in the crossbred herd at Beltsville.

Tables are presented showing the average butterfat production, the standard error of the mean, and the coefficient of variability of several registered and crossbred groups concerned with the experiment. All figures are based on actual first lactation butterfat records.

Four groups of registered Holsteins and Jerseys not connected with the crossbreeding project are used to establish a normal variation for butterfat production. The coefficients of variability for these ranged from 12.75 to 16.28 per cent. Five groups of registered foundation cows for the crossbreeding experiment showed coefficients from 10.25 to 21.36 per cent. The coefficients of variability of the registered daughters and their dams of the three principal sires used in the crossbreeding ranged from 11.82 to 22.59 per cent.

The first 42 two-breed crossbreds to complete first lactation records averaged 589.5 lb. of butterfat, with a coefficient of variability of 9.71 per cent. The first 16 three-breed crossbreds averaged 630 lb. of butterfat with a coefficient of 7.92 per cent. These figures seem to indicate that the crossbred groups exhibit unusual uniformity in butterfat production.

P12 Progress Report on Cross-breeding of Dairy Cattle at Beltsville.

M. H. Fohrman, Bureau of Dairy Industry, U. S. Department of Agriculture.

P13 A Case of Intersex in Dairy Cattle.[1] W. W. YAPP, University of Illinois.

The purebred Brown Swiss bull calf which was the subject of this study appeared normal and was sold as a breeding animal, to be delivered in 90 days. Just prior to shipment, the animal was examined carefully by the University herdsman, who thought he detected a slight enlargement of the scrotum. A veterinary confirmed this observation. Assuming the condition to be due to a slight injury, shipment was delayed 2 weeks and treatment to reduce the part begun.

After the condition failed to respond to treatment, it was concluded that a slight hernia was involved and plans were made to reduce the condition by surgery. The first incision of the operation revealed that the individual could not be a normal male. The animal was sacrificed to study the condition.

Inspection revealed a normal male reproductive system except that the testicles and accompanying ducts were absent. A normal female reproductive system, except for a portion of the vagina and vulva, also was found. The size of the bi-cornus uterus was essentially that expected in a normal female of a similar age and weight. The identity of the organ was indicated by the presence of numerous cotyledons.

Histological examination of the various tissues confirmed their identity except that the organ assumed to be an enlarged ovary actually was a vestigial testicle. The presence of this testicular tissue and its ability to secrete testicular hormones accounted for the secondary sexual characteristics of the animal and explains why he was assumed to be a normal male.

An attempt was made immediately to locate other cases similar to this one and also to locate males that had been sold as breeders but had failed to produce progeny. No other cases of intersex similar to the animal described have been found. Two males that were not capable of reproduction were located. Post mortem examination indicated the possibility of intersex but did not show the same development of reproductive organs of both sexes as found in the animal described.

One Brown Swiss bull that had produced several (three definitely known) sterile male progeny, two from the same cow, was located and purchase arranged for. All sterile animals had been sold for beef and could not be examined posthumously. Casual examination by breeders indicated some probability that they may have been cases of intersex. The owner of the bull, fearing that a further test of the breeding qualities of his bull might reflect on his herd, disregarded his promise and sold the animal for beef, thus making it impossible to test further the nature of the sterility found in the progeny that might have resulted from his service.

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[1] Members of the Department of Animal Pathology, University of Illinois, especially Dr. L. E. Boley, were very helpful in making post mortems and in identifying tissues.

P14 Dairy Cattle Improvement Work of the Indian Agricultural Research Institute—India.[1] J. D. S. KUMARAN, Department of Dairy Husbandry, University of Missouri.

The Government of India Agricultural Research Institute maintains the Sahiwal herd at New Delhi and the Tharparkar herd at Karnal. The paper deals with the work done at Karnal since 1923. The original home of the Tharparkar cattle is the semi-desert regions of the province of Sind.

The average yield per cow per day was 5 lb. in 1923. Line-breeding, selection, feeding, and sound herd management have resulted in a consistent rise in the milk yield. In 1930 the yield was 8.7; in 1935, 13.4, and in 1945, 20.5 lb. per cow per day.

The calves all are weaned at birth and are fed with standardized milk; the bull calves are fed for 6 months and the heifer calves for 8 months from birth. The average birthweight of the male calves is 54 lb. and the female cálves 48 lb. The birth weights of the males increase in a very steady manner as the weight of the dams increase. In the case of the female calves, the increase varies, though the maximum weight is found in the maximum weight class of the dam. The data further show that as the weight of the dam increases, the weight of the calves at birth also increases.

An analysis of 1,202 gestations reveals an average gestation period of 288.4 days, with the male calves taking a longer period than the females.

Heifers are bred after they attain a body weight of 600 lb. The average age of such heifers at first estrus was 23 months and 13 days, with a body weight of 609 lb. The average at first estrus after calving was 34 months, with a body weight of 733 lb. Tables give the different interestral periods for the different age classes.

Some cows have yielded more than 10,000 lb. in 306 days, and Tiqabdi 336 in 1946 yielded 10,369 lbs., which is the highest for the Tharparkar breed.

Pregnant heifers and cows from a fortnight to a month before calving are massaged and milked prenatally, which seems to have a beneficial effect in avoiding milk-fever and mastitis.

The Tharparkar cattle resist high diurnal temperatures and extreme variations in temperature. They have long loose dewfolds with sweat glands. During the hot summer days the temperature at Karnal reaches a maximum of 118° F., and the animals do not suffer any adverse effects from such high temperatures.

P15 The Use of the Pen Barn as a Means of Mastitis Control. P. L. KELLY, D. F. BREAZEALE, G. S. HARSHFIELD, AND A. B. HOERLEIN, South Dakota State College.

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[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1052.

From November 28, 1945, to April 1, 1946, dairy cows of comparable quality were divided into two groups. Twenty-seven remained in a regular stanchion barn and 13 were placed in a loafing shed. On the basis of strip cup tests, 11 cows in the stanchions and 4 cows in the pens had attacks of mastitis. These data indicate a gain in the control of a mastitis infection by the use of a pen barn, in spite of the fact that stanchions which were exposed to possible drafts were not used.

From November 28, 1946, to April 1, 1947, all cows were kept in stanchions. On the basis of the strip cup test, only 6 cows of the average of 43.4 actually in milk had attacks due to causes other than injury. All of these cows had had previous infection with *Streptococcus agalactiae.* This was true even though all of the stanchions were used.

Apparently during periods of heavy infection with the usual organisms which cause mastitis, the use of a loafing shed where cattle can move at will and lie on a warm floor is an important means of getting the infection under control. However, after the amount of infection has subsided, the use of a pen barn for mastitis control is not so important.

P16 Report on Attempts to Prevent Midsummer Slump in Milk Production by Hay Feeding. D. M. SEATH AND G. D. MILLER, Louisiana State University.

A double-reversal experiment was conducted in 1946 with 20 cows over a period of 60 days to determine the advisability of feeding hay during warm summer months while cows are on pasture. This trial was conducted as a direct result of a previous experiment which showed that cows on hot days often spend less than 2 hours grazing between morning and evening milkings. In the present trial at around 11 a.m. daily, cows were fed mixed white Dutch clover and canary grass hay in racks located in the shade. Two levels of hay feeding were tested. In one case the cows were limited to 0.5 per cent of body weight daily and in the other they were fed all they would clean up. Hay consumption averaged 4.69 lb. per cow daily under the first plan and 5.54 lb. daily for the second.

Milk production records showed that the full hay-feeding plan produced significantly more milk than when no hay was fed. The increases averaged 0.1 and 1.0 lb. milk per day per cow, respectively, for the two test groups. This increase would not be economically profitable. On limited hay the average increase was slightly less (0.4 and 0.1 lb. more per cow daily), again making the practice unprofitable under the conditions prevailing, which included excellent permanent pastures stimulated by abundant rainfall. Weather conditions were considered much better than average for pasture growth and may have influenced the results of the experiment.

P17 The Influence of Cracked Soybeans and Soybean Hay on the Flavor and Quality of Milk. Erle E. Bartley and C. Y. Cannon, Iowa State College.

The Iowa dairy industry long has been troubled with the development of oxidized and other undesirable flavors in milk. Since soybeans are the chief source of homegrown protein in Iowa, some of these flavors were attributed to the feeding of soybeans on the assumption that soybeans increase the proportion of unsaturated fatty acids in butterfat and thereby would increase the susceptibility of milk to the development of oxidized flavor.

Tests were made to determine the flavor effect on milk when cows were fed four different rations, two containing soybean hay and two alfalfa hay. The two grain mixtures fed were similar, except that one contained cracked soybeans and the other linseed meal. The rations were assigned to the cows within each group and between groups at random according to a latin square design so that each cow was fed each of the four rations in succeeding periods of 5 weeks each. The milk was collected in glass, well-tinned, and rusted tinned containers.

Neither soybeans nor soybean hay as such seemed to affect adversely the flavor of milk; in fact, the milk scores were slightly higher when soybean hay was fed than when alfalfa hay was in the ration. This difference probably was due to a difference in quality of hay rather than to the variety. Contrary to results in a previous trial, the rusted tinned containers adversely affected the flavor of the milk regardless of whether the milk was produced from animals fed soybeans or not. This result was not unexpected, for the exposed iron in the rusted tinned containers easily could act as a catalyst accelerating the oxidation of the fat.

P18 The Value of Adding Ground Alfalfa Hay to the Concentrate Mixture Fed with Prairie Hay in Rations for Dairy Cows. A. H. Kuhlman and H. W. Cave, Oklahoma A. & M. College.

Economical and maximum milk production is possible only when balanced rations are fed. Because of critical shortages of high protein concentrates in recent years, many farmers who use prairie hay or any other non-legume roughage often have been unable to secure sufficient quantities of cottonseed meal or similar feeds to prepare suitable home-mixed dairy concentrates.

Two feeding trials have been completed in which ground alfalfa hay was used to replace part of the cottonseed meal and other feeds in a concentrate mixture suitable for use with prairie hay. In each trial two lots of six cows each were fed the control and experimental mixtures in three 30-day periods according to the double reversal plan commonly used in feeding experiments with dairy cows. One cow was removed from the experiment during the second trial because of a very sudden decline in milk production. Results, therefore, are based on the records of 23 cows.

Prairie hay was fed to each cow at a level such that the hay supplied an amount of total digestible nutrients equivalent to her requirements for body maintenance. The control concentrate mixture contained 350 lb. ground no. 2 yellow corn, 200 lb. ground oats, 200 lb. wheat bran, and 250 lb. cottonseed meal. The experimental mixture consisted of 245 lb. ground no. 2 yellow corn, 140 lb. ground oats, 140 lb. wheat bran, 175 lb. cottonseed meal, and 300 lb. ground alfalfa hay. On the basis of Morrison's values for the average composition of feeds, the control mixture contained 15.5 per cent digestible protein and 75 per cent T.D.N., while the experimental mixture contained 14.0 per cent digestible protein and 68 per cent T.D.N.

The two rations were equally efficient in maintaining body weights and milk production. All cows were weighed daily during the experiment. The average daily weight of the 23 cows was 1,085 lb. while fed the control ration and 1,102 lb. when fed the experimental ration containing the ground alfalfa hay. The average daily yield of 4 per cent fat-corrected milk per cow in the two trials was 29.67 lb. on the control ration and 29.50 lb. on the experimental ration.

For each 100 lb. of 4 per cent F.C.M. produced, the cows consumed 50.76 lb. of concentrates and 59.66 lb. of prairie hay when fed the control ration and 56 lb. of concentrates and 60.37 lb. of prairie hay on the experimental ration.

Ground alfalfa hay as fed in this experiment replaced 30 per cent of the concentrates in the control ration and effected a saving of 22.8 per cent of the cottonseed meal required. Each 100 lb. of alfalfa hay fed in the experimental ration replaced 24 lb. corn, 13.8 lb. oats, 13.8 lb. wheat bran, and 17.2 lb. cottonseed meal. At current feed prices ground alfalfa hay can be used advantageously in a prairie hay ration for dairy cows.

P19 The Partition of Lipids by the Lactating Dairy Cow as Influenced by Certain Mineral Supplementation. G. M. WARD AND J. T. REID, Rutgers University.

The effects of calcium and other mineral supplements upon the metabolism of lipids were investigated during the first 5 months of lactation of eight Holstein and four Guernsey cows. The study was initiated after the observation was made that cows receiving supplemental calcium excreted a much smaller proportion of the crude fat (ether extract) intake by way of the feces than did their controls not receiving supplemental calcium.

An oxidative method was devised for the estimation of various lipid fractions in feeds and feces, employing chloroform as the solvent, doucil (an artificial zeolite) as the phospholipid adsorbent, and dichromate as the lipid oxidant. The procedure gave satisfactorily reproducible results and an efficient recovery of various classes of lipids.

All animals excreted similar proportions of the total lipid intake regardless of the mineral supplement(s) received. Although increased intakes of calcium tended to spare the fraction consisting of neutral fat, sterols and waxes and the fraction containing chiefly phospholipids, there was a corresponding increase in the excretion of the fraction comprised of soaps and free fatty acids, indicating that mineral supplementation was without effect upon the total lipid economy of milking cows but was qualitative rather than quantitative in effect. The observed differences relative to the smaller proportion of ingested ether extractives lost in the feces of animals receiving supplemental calcium were attributed to an increased formation of soaps, which were not measured in the feces of these animals because of the insolubility of soap in ether.

These results indicated that the estimation of crude fat by the commonly employed ether extraction procedure is apt to be misleading in experiments involving the analyses of dairy cow feces.

During the relatively short period of experimentation no apparent relationship existed between the efficiency of lipid use and the stage of lactation.

P20 Cobalt Tolerance in Young Dairy Cattle.

H. A. KEENER, G. P. PERCIVAL, AND K. S. MORROW, University of New Hampshire, AND G. H. ELLIS, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, New York.

Cobaltous sulfate was fed to young Holstein cattle at levels varying from 5 to 125 mg. of cobalt per 100 lb. of body weight per day. Certain levels of cobalt feeding were maintained for periods up to 34 weeks, although some animals actually received cobalt continuously for periods up to one year. One 2-year-old Holstein heifer was fed the equivalent of over 230 mg. of cobalt per 100 lb. of body weight per day for 28 days.

The first effects of excessive cobalt consumption observed were increased hemoglobin, red blood count, and packed red cell volume. These effects usually were observed only after a considerable period on the higher levels of cobalt intake. On the highest levels a few animals went off feed, consumed less water, and exhibited a rough hair coat and an unsteady gait.

It would appear that growing dairy cattle may consume about 50 mg. of cobalt per 100 lb. body weight per day without ill effects. This value is well over one hundred times the amount normally consumed from commercial cobalt-supplemented dairy rations.

P21 Phosphorus Metabolism Studies. I. Secretion and Partition of Dietary Radioactive Phosphorus in the Milk of the Dairy Cow.

C. L. Comar, R. B. Becker, P. T. Dix Arnold, W. A. Krienke, and George K. Davis, Florida Agricultural Experiment Station.

Separated phosphorus 32 from the United States Atomic Energy Commission is being used in studies of phosphorus metabolism and particularly phosphorus secretion in milk. Radioactive phosphorus was administered quantitatively in feed as disodium phosphate solution. Measurements were made with a dipping type Geiger-Mueller tube directly on the milk when there was sufficient activity, or on an ash solution when concentration was necessary.

The phosphorus was given in the afternoon feeding as a single dose to each Jersey cow selected from the Station herd. Milk samples were taken the following morning and at subsequent regular milkings twice daily. The highest concentrations of radioactive phosphorus always were found in the first three milkings, decreasing regularly thereafter. Activity has been observed in the milk 17 days after administration of the single dose. Present data, considered as preliminary, may be summarized as follows: With three animals near the peak of lactation (31 to 44 days), and producing 33 to 22 lb. of milk daily, 15.1 to 12.2 per cent of each phosphorus dose was secreted in the milk during the following 10 days. Three other cows in mid-lactation (129 to 196 days), producing 20.8 to 16.2 lb. of milk daily, secreted 8.1 to 7.3 per cent of the dosage in the milk during 10 days.

Fractions of the milk in which none of the radioactive phosphorus was found were the butterfat, the ether extracts of the buttermilk, and the lactalbumin. The casein fraction, whether prepared by acid precipitation followed by washing or purified according to the Hammarsten procedure, contained an average of 25 per cent of the radioactive phosphorus present in the skim milk from which it was obtained. The sum of the casein and acid serum values agreed with the activity as measured in the original skim milk.

P22 Feeding Value and Digestibility of Dehydrated Sweet Potatoes.

L. L. Rusoff, D. M. Seath, and G. D. Miller, Louisiana State University.

Within the past few years dehydrated sweet potatoes have been used more and more in the southern states as a carbohydrate feed for livestock. The nutritive value of dehydrated sweet potatoes for dairy cows has been investigated at the Louisiana Station. Three feeding trials and four digestion trials were run on dehydrated sweet potatoes representing products produced in 1944, 1945, 1946, and 1947. The percentage composition on the dry basis of the 1947 dehydrated sweet potatoes, which is a typical analysis, showed 85.35 per cent dry matter, 4.17 per cent crude protein, 1.15 per cent crude fat, 4.46 per cent crude fiber, and 84.44 per cent N.F.E.

In feeding trials with dairy cows, dehydrated sweet potato meal was found to be equal to approximately 88 per cent of yellow corn. Milk production and butterfat yields favored yellow corn meal by 4.05 and 4.55 per cent, respectively. Daily milk production for cows on the dehydrated sweet potatoes was 24.9 lb. compared with 25 lb. for cows fed ground snapped corn, with allowance made for cob and shuck. Dehydrated sweet potatoes made from whole tubers were palatable, while a product made from culled, bruised, sectioned potatoes and peelings was slightly unpalatable.

Digestion trials showed that dehydrated sweet potatoes contained from 71 to 81 per cent T.D.N. The N.F.E. was highly digestible, while the other constituents showed an apparent lack of digestibility. Since the N.F.E. made up over 84 per cent of the dry matter of the dehydrated sweet potatoes, the other constituents would have but a small effect on the T.D.N.

Dehydrated sweet potato meal is of value in the dairy ration not only because of its high digestibility of carbohydrate but also because of its high carotene content.

P23 Studies on the Globulins of Bovine Colostrum. I. Isolation and Properties of a Water Soluble Globulin.[1] R. G. HANSEN AND P. H. PHILLIPS, Department of Biochemistry, University of Wisconsin.

Globulin protein has been isolated from colostrum by precipitation with alcohol in the cold, under carefully controlled conditions of alcohol concentration, pH, protein concentration, salt concentration, and temperature. This material probably contains the euglobulin and pseudoglobulin of Howe. A water soluble globulin also has been isolated from colostrum by ammonium sulfate fractionation according to the method of E. L. Smith. This material was found to be homogenous in the ultra centrifuge and about 95 per cent homogenous when subjected to electrophoresis.

Amino acid determinations have been made on this protein by microbiological assay. The analyses indicate a striking similarity in amino acid composition to purified human serum gamma globulin. A difference from the casein and the β-lactoglobulin of normal milk was indicated. In agreement with the findings of Smith, the most highly purified globulin contains carbohydrate material. The purified globulin has been fed to new-born calves and was found to increase the gamma globulin fraction of the blood serum. Electrophoretic studies indicate a similarity between these two proteins. Attempts to stimulate development of the serum gamma globulin fraction of the deficient calf, by seeding with an intravenous injection of the colostrum globulin at a level of approximately one twenty-fifth of the normal amount of gamma globulin present, were unsuccessful.

[1] We wish to express our appreciation to Prof. J. W. Williams for his cooperation and to Messrs. L. A. Henderson and R. L. Potter for amino acid analyses.

P24 Studies on the Globulins of Bovine Colostrum. II. The Absorption of Globulins by the Young Calf.[1] R. G. Hansen and P. H. Phillips, Department of Biochemistry, University of Wisconsin.

Electrophoretic measurements on the serum of calves fed colostrum and on the serum of colostrum-free calves have been extended. In agreement with existing data, there is a marked increase in the slow-moving globulins (gamma globulins) of the young calf upon the ingestion of colostrum. If the new-born calf is given only normal milk, followed after 36 hours by colostrum, no increase in these serum protein fractions is noted.

An enzyme preparation from the gastric mucosa and chyme of a normal calf fed with colostrum was partially successful in preventing the increase of globulins of the blood serum in new-born calves.

If calves were raised on a ration free of colostrum, the blood serum remained low in its content of the gamma globulins until the calf was about 8 weeks of age, at which time the gamma globulin concentration approached a normal value.

Colostrum placed directly by operative technique into the small intestine of a gamma-globulin deficient calf at 2 weeks of age resulted in no measurable increase in the blood serum globulins.

These results suggest that a probable combination of increased digestive activity and variation in the permeability of the gut is responsible for the above observations.

P25 Tocopherol Levels in the Colostrum and in the Early Milk of the Dairy Cow. D. B. Parrish, G. H. Wise, and J. S. Hughes, Kansas Agricultural Experiment Station.

The tocopherol content of colostrum and of early milk from dairy cows of four different breeds was determined for the first four and the eighth days postpartum. Wide individual differences were observed. In all cases in which cows had the usual dry-rest period, with or without dietary tocopherol supplement, the average rate of decrease during the colostric period followed roughly an exponential curve with approximately the same slope for all individuals. Supplements of 500–1,000 mg. of tocopherol starting one month prepartal increased the tocopherol content of first colostrum 30 to 50 per cent above that in the colostrum of cows receiving no supplement; 10 g. of tocopherol per day increased the tocopherol in colostrum four to five times above the level for unsupplemented cows.

During the first four days, a 100-lb. calf fed at the rate of 10 lb. of colostrum daily would receive from its dam on an unsupplemented ration 28 to 86 mg. of tocopherols; from dams supplemented with 500 to 1,000 mg.

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[1] We wish to express our appreciation to Professor J. W. Williams for his cooperation and to Dr. C. K. Whitehair for performing the operation.

of tocopherol daily, 61 to 185 mg.; and from dams supplemented with 10 g. tocopherol daily, 205 to 373 mg. Ingestion of colostrum caused a rapid increase in the tocopherol level in the blood serum of the new born calf, being most marked when prepartal intake of tocopherols by the dam was high. The nutritional significance of tocopherols in the diet of the calf has not been established.

P26 Action of Bacterial Filtrates Infused into the Mammary Gland.

MAX L. DAWDY AND W. E. PETERSEN, University of Minnesota.

Bacterial-whey filtrates have been infused via the teat canal into the mammary gland in an attempt to find the causative mechanism in bovine mastitis. The pathogenic organisms, two *Staphylococcus* species, three species of the coliform group, and one culture of *S. agalactiae* in pure culture have been obtained from various acute and chronic mastitic quarters. Fresh whey from casein precipitation with rennet, the only medium found non-irritating to the tissue, rendered sterile by means of the Seitz filter, was inoculated with the desired culture and incubated 24, 48, and 72 hours at 37° C. The sterile filtrate was obtained by the Seitz filter and infused into the mammary gland in quantities of 2.5, 5, and 10 ml.

The filtrate from the coliform groups, when infused, results in all the manifestations of acute mastitis, with equal degree, regardless of volume infused or incubation time. A direct correlation with incubation time results with the staphylococcus, and no reaction results in the case of the filtrates of *S. agalactiae*.

Intramammary infusion of the filtrates into a large number of normal quarters consistently has produced the following manifestations: (a) Hardness and swelling of the quarter in 1 to 2 hours. (b) Clots in the milk in 2 to 4 hours, with the infused quarter up to twice its normal size. (c) Shivering, humped back, and pilo-erection for 2 to 4 hours, followed by body temperature increases of 4 to 6° F., usually reaching a peak in 6 to 8 hours. Acute inflammation of the quarter also occurs. (d) Rapid fall of body temperature to about normal at 12 hours. (e) Drop in milk production. (f) Decrease in swelling and inflammation at about 24 hours, but palpable thickness of the infused quarters with physically abnormal milk persisting about 4 days.

Although the effective product of the filtrate yet is undetermined, its action has similarity to bacterial toxin reactions. In mice of 8 weeks of age, an M.L.D. of 0.6 ml. of the coliform filtrate was determined, but a lethal dose of the staphylococcus filtrate has not been reached. Red blood cells are hemolyzed almost completely. The clots obtained have a physical resemblance to blood fibrin clots. The milk drawn 1 to 2 hours following infusion can be shown to react to the anti-coagulation mechanism of blood by citrate and subsequent coagulation with calcium chloride, suggesting that the clots found in mastitis may be the result of change in vascular permeability to blood proteins.

P27 The Effect of Intramammary Treatment for Mastitis upon Milk Production.[1] ERIC W. SWANSON AND H. A. HERMAN, Department of Dairy Husbandry, University of Missouri.

A major consideration in the intramammary treatment of lactating cows infected with streptococci but not showing clinical mastitis is the effect such treatment will have upon milk production. Since the use of sulfanilamide in oil and penicillin in water seemed to cause the least reaction of any mastitis-treatment materials injected into the udder, the effect of these upon milk production has been studied in the University of Misouri herd. The average daily milk production was computed for 3 days prior to treatment, during treatment, and for 7 days after treatment, with critical comparisons being made between the pretreatment average and the average production during treatment. Sixty-three series of treatments with sulfanilamide in iodized mineral oil, injected once daily for 4 days, were studied; 19 series of treatments with penicillin in distilled water, injected twice daily for 2 days, were included.

Treatment with penicillin did not cause a significant change in milk production. The average change was a decrease of 1.56 lb. per day, but 10 of the 19 cows increased in production during treatment. The sulfanilamide treatments were analyzed according to the number of quarters treated, amount of milk produced, and size of injection. In all groups a significant decrease in milk production occurred, and the pretreatment level was not attained until about 7 days following cessation of treatment. The average decreases in daily production when one, two, three, or four quarters were treated were, respectively, 2.59, 3.07, 3.43, and 3.23 lb. Differences due to number of quarters treated were not significant. This would indicate that the cause of the decline may not be a simple mechanical or chemical effect upon the milk secreting tissue but may be due to greater effects, possibly of a systematic nature which affects milk secretion of the entire udder when one or more quarters are infected. Decreases did not vary directly with production, averaging 1.71 lb. for 1- to 20-lb. producers, 3.71 lb. for 21- to 40-lb. producers, and 2.40 lb. for producers above 40 lb. The high-producing cows seemed to reach their pretreatment level faster than low-producing cows following cessation of treatment. Differences between decreases from 50, 60, and 80 ml. injections were not significant, although five of six cows given 80 ml. injections declined more than when 60 ml. were injected.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1047.

P28 The Use of Penicillin in the Treatment of Mastitis vs. Natural Recovery. D. F. BREAZEALE, P. L. KELLY, G. S. HARSHFIELD, AND A. B. HOERLEIN, South Dakota State College.

These experiments have been in progress since May, 1945. Previous to this time various other medications had been used in this herd. Approximately one-half of the cows had mastitis when the penicillin treatments were begun. Since penicillin could be obtained only in very limited quantities, 10 cows showing the most extensive indurations, and therefore thought to be least likely to yield to treatment, were isolated from the main herd. About 3 months later they were returned to the main herd and treated.

Diagnostic methods used were: (a) udder palpations, (b) daily strip cup tests, (c) microscopic examination of incubated samples for types of bacteria and numbers of leucocytes, and (d) growth of organisms on blood agar followed by identification of streptococci and coagulase tests on staphylococci.

With the exception of a few of the first cases, the dosage of penicillin was standardized at 100,000 Oxford Units per treatment per quarter. This was dissolved in 100 ml. of sterile distilled water and was infused into the udder by gravity. Eighty-seven quarters were treated during the first year and 28 during the second year. Quarter samples were examined at monthly intervals during the first year and at quarterly intervals during the second year. The progress of all treatments was followed bacteriologically a few days after the injection of penicillin and at the regular samplings as long as the cow remained in the herd. The data reported below do not include the work done since January 1, 1947.

Of 73 quarters treated for *Streptococcus agalactiae*, 77.7 per cent became negative to this organism for one month or longer; however, 50 per cent of these "cured" cases again became positive to this organism at varying time intervals, averaging 5.9 months.

When the treatments for *S. agalactiae* are divided into cases of recent infection (found on one examination only) and those of longer duration, the data show 90 per cent "cures" in the first group and only 59 per cent in the latter.

S. agalactiae were found in routine samplings of 37 quarters not treated. No attempt was made to select these cases. The majority of these occurred during a period of about 6 months (the first half of the second year) in which no penicillin was used; a few cases occurred during the first year. Of these 37 quarters not treated, 43.3 per cent became negative to *S. agalactiae*. When these cases are divided into those which were positive only once in routine examinations and those of longer duration, 65.2 per cent became negative in the first group and only 7.1 per cent in the latter group. This suggests that in some of the cases in which *S. agalactiae* was found the infection was not firmly established.

Eighteen quarters positive for staphylococci were treated with penicillin. Some of these quarters were treated several times. Only 50 per cent of these became negative to the organism, and 44.4 per cent of these "cures" again were positive after an average time elapse of 1.5 months.

Staphylococci were found in routine samplings of 93 quarters not treated; 76.4 per cent of these became negative to this organism. When these cases are divided into those in which the organism was found in only one sampling and those of longer duration, 83.8 per cent in the first group became negative and 47.4 per cent in the second group. Apparently many of these cases were not firmly established infections.

P29 The Use of Quaternary Ammonium Compounds for Washing the Udders of Dairy Cows.

ROBERT H. KEITH AND PAUL M. REAVES, Dairy Husbandry Department, Virginia Polytechnic Institute.

Recently a number of quaternary ammonium compounds effective in the sterilization of dairy utensils have been developed. In order to study their value in washing the udders of dairy cows just prior to milking, these products were compared with chlorine and with a plain water wash. The results indicate that they are effective as sanitizing agents. They also appear not to cause the hands of the milker or the teats of the cow to chap, as chlorine often does when used in hot water and especially in cold weather. Emulsept, Hyamine T, B T C, and Roccal were used in these trials.

P30 The Effect of Reduced Feed Intake on Mammary Growth and Lactation.

J. F. SYKES, T. R. WRENN, AND S. R. HALL, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

The effect on mammary growth and subsequent lactation of restricting a group of rats to 70 per cent of the feed consumption of litter mates fed *ad libitum* during the growing period and through pregnancy has been studied. At parturition mammary glands were obtained from half of each group and the remaining half allowed to nurse litters for 21 days on full feed to correlate observed mammary growth at parturition with subsequent lactation.

The results obtained indicate that feed restriction during the growing period and pregnancy reduces the size of the mammary gland out of proportion to the reduction in body size. However, if access to sufficient feed is allowed after parturition, a rapid growth takes place and the glands actually may lactate more efficiently than those which were better developed at parturition.

P31 Further Studies on the Effect of Vitamin D and of Parathyroid Extract, "Paroidin", on the Blood Changes of Normal and Milk-

Fever Cows at Parturition. J. W. Hibbs, W. D. Pounden, and W. E. Krauss, Ohio State University.

Previous experiments have shown that the administration of from 2 to 5 million units of vitamin D daily, in the form of irradiated dry yeast or "Viosterol," for from 2 to 4 weeks prepartum will increase serum calcium and phosphorus at parturition. However, the increases thus obtained are nullified completely in both normal and milk-fever cows within 12 hours postpartum, or before milk fever usually occurs. No lowering of milk fever incidence could be demonstrated in these experiments.

The possibility was recognized that the previous vitamin D feeding periods might have been too long. The feeding of vitamin D for extended periods of time is known to suppress parathyroid activity. Experiments in which the increases in serum calcium and phosphorus were followed closely after the initiation of vitamin D feeding to non-parturient cows showed that a peak is reached in from 10 days to 2 weeks. Therefore it was decided to study the effect on the blood serum calcium and phosphorus of feeding massive doses of vitamin D (10 million units daily) beginning 5 to 7 days prepartum. The serum calcium and phosphorus thus would be increasing at parturition and possibly the calcemic action of vitamin D would not be masked by parathyroid suppression.

Results to date, although the numbers are few, indicate that the serum calcium and phosphorus were increased at parturition, and that the usual postpartum decrease was slight as compared to the control cows when this system of vitamin D supplementation was used.

Parathyroid extract, "Paroidin", when injected subcutaneously into non-parturient cows in single doses of from 2 to 3 thousand units, regularly caused an increase of from 1.5 to 2 mg. per 100 ml. in serum calcium and phosphorus, reaching a maximum in from 15 to 20 hours after injection.

When 3, 4, and 5 thousand units, respectively, of "Paroidin" were administered to three cows with early milk fever symptoms, no benefit could be detected. A fourth cow was injected with parathyroid extract (4,000 units) and also was fed 10 million units of vitamin D on the day before parturition and again at the time of the injection. This cow was exhibiting mild early milk fever symptoms at the time of injection. She did not go down with milk fever, and the serum calcium remained constant at about 6.50 mg. per 100 ml. for about 24 hours. The serum calcium level was found to be normal after 48 hours.

It is believed that the failure to mobilize calcium from the skeletal reserves in milk fever is due either to a failure of the parathyroid glands to secrete sufficient hormone or to the situation in which sufficient hormone is secreted but is rendered temporarily inactive by some metabolic condition in the tissues at parturition.

Vitamin D yet may prove to be beneficial in the control of milk fever if a suitable dosage administered at the proper time can be determined.

P32 The Effect of the Quality and Quantity of Feed Prepartum and Postpartum upon Blood Glucose and Blood Acetone Bodies.

J. C. SHAW AND G. M. CAIRNS, Maryland Agricultural Experiment Station.

Thirty cows were divided into six groups and placed on an experiment beginning 16 weeks prepartum and ending 4 weeks postpartum. All of the cows received 140 per cent of requirements during the first 8 weeks, 180 per cent during the second 8 weeks, and either 50 per cent postpartum for 3 weeks followed by 100 per cent or 100 per cent for the entire 4 weeks postpartum.

The rations fed were designed to study the influence of the fat, protein, soluble carbohydrate, and vitamin A content of the prepartal ration upon the postpartal level of blood glucose and blood acetone bodies.

While several of the cows developed a marked hypoglycemia, none exhibited ketonemia. The data provide conclusive evidence that a vitamin A deficiency in dairy cows does not produce ketosis even on fasting. A limited T.D.N. intake of 50 per cent postpartum produced hypoglycemia most frequently and of a greater severity in those cows in which the total T.D.N. requirements were greatest. The data also demonstrated that a deficiency of blood glucose alone is insufficient to produce ketosis. High soluble carbohydrate rations fed both prepartum and postpartum did not aid in preventing hypoglycemia. A high fat-low protein ration during the prepartal period, followed by limited feed intake postpartum, did not produce ketosis and did not result in any greater hypoglycemia than some of the other treatments.

The summarized results differ in some respects from those obtained at the University of Connecticut, where both hypoglycemia and ketonemia were observed (unpublished). However, in these experiments energy intake was maintained at 60 per cent of requirements for 2 weeks preceding parturition and at 50 per cent following parturition. Likewise, the latter animals may have been in somewhat better condition. While the blood acetone bodies may exhibit an inverse relationship to the blood glucose, such relationship does not hold under all conditions. This indicates that while energy intake undoubtedly plays a rôle in the development of ketosis, it is not the only factor involved and, indeed, may not be the most important factor.

P33 Vitamin A Deficiency in Dairy Cattle on Rations Containing Ground Raw Soybeans.

J. C. SHAW, Maryland Agricultural Experiment Station, AND L. A. MOORE AND J. F. SYKES, Division

of Nutrition and Physiology, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

Twelve cows receiving a low-carotene timothy hay and a concentrate containing 40 per cent raw soybean meal for 4 months prepartum dropped calves showing signs and symptoms of a vitamin A deficiency. One cow exhibited incoordination and five retained their placentas. Four cows on a basal ration not containing soybean meal dropped two calves which appeared to be completely normal and two dead calves. There appeared to be no way of ascertaining whether the two calves died of a vitamin A deficiency. None of the four controls retained their placentas. All of the cows, both controls and normals, exhibited low levels of plasma vitamin A following parturition. The plasma vitamin A levels of the calves showed little or no increase after receiving colostrum milk from their dams for 5 days. The lowest plasma vitamin A levels were observed in calves whose dams received the soybean ration unsupplemented by vitamin A.

Five cows received the soybean ration for 4 months prepartum, supplemented by the feeding of 400,000 I.U. of a vitamin A concentrate for the last 4 weeks prepartum. Four of the five appeared to be normal at birth. The fifth appeared to be normal but died of bacteremia on the second day. The plasma vitamin A of the four calves increased markedly after receiving colostrum milk from their dams.

An additional experiment was conducted with calves to obtain further information on the possible detrimental effect of raw soybean meal when fed to dairy cattle on low levels of carotene intake. Four calves at 4 months of age were partially depleted of vitamin A on Moore's vitamin A-deficient ration, after which they received 32 μg. of carotene per day per pound of body weight. Two of the calves were continued on the basal ration and two received a similar ration to which raw soybean meal was added to the extent of 30 per cent. Blood plasma vitamin A was determined weekly and liver vitamin A was determined at the end of the experiment. The plasma vitamin A consistently was lower in the two calves receiving the ration containing the raw soybean meal, averaging 5.64 and 6.60 μg. per cent, whereas that of the control calves was 9.60 and 9.32. The liver vitamin A (wet basis) was 0.72 and 2.75 μg. per g., respectively, for the calves on the soybean ration and 9.07 and 9.22 μg. per g. for the calves on the basal ration.

P34 Values of Regular Corn Silage, Grainless Corn Silage and Ear Corn Silage in the Dairy Ration. K. M. Dunn, R. E. Ely, and C. F. Huffman, Michigan Agricultural Experiment Station.

Fifteen lactating cows depleted of unknown lactation factors were used in these studies. The method used to determine the relative feeding value of total digestible nutrients of various feeds was designed by Huffman (Jour. Dairy Sci., **21**: 101. 1938).

Results of the feeding trials indicated that grainless corn silage was equal to clover hay on a total digestible nutrient basis for milk production. When corn grain replaced grainless corn silage on a total digestible nutrient basis, there was a daily increase of 2.5 lb. of 4 per cent fat-corrected milk during the fourth 3-day period.

The replacement of part of the clover hay with ear-corn silage on a total digestible nutrient basis resulted in a significant increase in milk production. This increase reached its peak during the fifth 3-day period, at which time there was an average daily increase of 4.7 lb. of 4 per cent fat-corrected milk. The total digestible nutrients in ear-corn silage was equal to the total digestible nutrients in corn-and-cob meal for milk production.

When grainless corn silage plus corn-and-cob meal replaced regular corn silage, no significant change in milk production occurred. These data indicate the corn grain in corn silage is equal to dry corn grain stored in a crib for milk production.

Corn grain and ear-corn silage carried some unknown factor or factors that stimulated milk production. The clover hay used in these studies appeared to be low in these factors.

P35 Carotene Losses from Artificially Dehydrated Alfalfa and from Artificially Dehydrated Alfalfa Silage.[1] R. G. WASHBURN, W. E. KRAUSS, AND C. F. MONROE, Ohio Agricultural Experiment Station.

A study was made of the carotene losses from artificially dehydrated alfalfa meal and from the same material preserved as artificially dehydrated alfalfa silage.

In June, 1946, a field of alfalfa was divided into two parts. One part was field harvested, the crop immediately dehydrated in a commercial dehydrator, and then ground, sacked, and stored in a barn. The remaining part was ensiled with the addition of molasses. This silage remained in the silo until mid-December, 1946, when it was dehydrated, ground, and stored until the latter part of January, 1947.

The loss in carotene from the artificially dehydrated alfalfa meal, stored in the barn from July, 1946, to May, 1947, was 48 per cent. The alfalfa meal stored at the place of dehydration had a loss in carotene from July, 1946, to April, 1947, of 55.5 per cent.

The amount of carotene lost during the period that the alfalfa was ensiled and then dehydrated, *i.e.*, from June, 1946, until mid-December, 1946, was 30 per cent.

Although there was more carotene in dehydrated alfalfa silage than in stored dehydrated alfalfa meal, the physical characteristics of the dehydrated silage were such as to question the advisability of attempting to preserve carotene in this way for the production of commercial alfalfa meal.

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[1] The alfalfa meal and dehydrated silage were made and shipped to the Ohio Experiment Station by the Farm Bureau plant at Payne, Ohio.

P36 Silage Density: Effect of Pressure and Crop Condition.

A. E. Perkins and R. G. Washburn, Ohio State University.

Sixteen series of silo presses with 12 to 18 in each series have been filled and carried through an observation period of about 35 days. The crops used were corn or meadow crop mixtures in which alfalfa, clover, and timothy predominated.

Usually one material at three different degrees of dryness was cut uniformly. Weights of crops which supplied about the same amount of dry matter were packed lightly into the silo presses.

The initial depth was recorded and a series of weights, ranging from 1 to 16 lb. per sq. in. of silage surface simulating the pressures thought to prevail at different levels in farm silos, was applied to the material of each degree of dryness. These weights remained in place throughout the experimental period. Depth readings were taken daily at first and then at longer intervals. The occurrence of any leakage was noted.

The cross sectional area of the silo presses and the dry matter of the ensiled material were determined in advance. The volume and the density of the ensiled material as grams per cubic centimeter were calculated for each of the depth measurements and grouped for study. The following relationships were shown:

1. A greater total weight, but a smaller weight of dry matter, could be stored in a given space with a green than with a drier crop.

2. The volume shrinkage or increase of density was greater and more rapid with the green or wetter crops than with those which were drier or more mature.

3. The greatest increase in dry matter density was obtained in the case of crops of 35–40 per cent dry matter. The greatest absolute density of dry matter, however, was obtained in the driest group, that above 50 per cent dry matter.

4. When the results at all degrees of dryness were averaged to study the effect of varying pressure, the density increase was rather slow and small at 1 lb. per sq. in. pressure, less than 1 per cent for 1 day and only 9 per cent in 30 days. At 16 lb. per sq. in., the density increase was 53 per cent for 1 day and 93 per cent for 30 days.

5. Leakage from the ensiled material began when the density approached 0.9 g. per cc., or about 56 lb. per cu. ft.

6. The material remaining after extensive leakage at the higher pressures attained a density of 1.0 g. per cc., 62.5 lb. per cu. ft. or higher.

7. The dry matter density of silage at low-to-medium pressures is equal

to that of commercially baled hay, while silage at the higher pressures may contain twice as much dry matter per unit volume as baled hay.

8. Contrary to the prevailing idea, the difference in density between corn silage and hay crop silage at corresponding dry matter and pressure levels was small.

P37 The Comparative Feeding Value of Corn Silage and of Corn-treated Meadow Crop Silages, with and without the Addition of Dilute Acetic Acid. C. F. MONROE, A. E. PERKINS, C. E. KNOOP, AND R. C. THOMAS, Ohio State University.

Three silages were compared in a double reversal feeding trial which consisted of three periods of 35 days each. The silages were fed in six different sequences to a like number of outcome groups, each composed of one Jersey and two Holstein cows. With the exception of the kind of silage fed, all the cows received the same rations and general treatment.

All the silages were made by a forage crop harvester which cut and chopped the crops in the field. The corn silage was of moderately good quality, made from a grain variety of hybrid corn. Both of the meadow crop silages were made from the same mixture, consisting of 72 per cent timothy, 22 per cent clover and alfalfa, and the remainder of weeds and volunteer wheat. To both of these silages approximately 100 lb. of ground corn were added per ton of material as it was being blown into the silo. In addition, one of the silages was sprayed with a 12.5 per cent solution of acetic acid at the rate of 5 gallons per ton. This small addition of acetic acid, amounting to 0.25 per cent, was made in the hope of controlling the original fermentation and thereby improving the quality of the product. The silage resulting from this treatment was of good quality, but only slightly better than the one which received the corn treatment only. Both the meadow crop silages and the corn silage contained approximately 28 per cent dry matter.

The feed intakes were practically the same on all three of the silage rations. Likewise, the productions showed no significant differences. The average productions of 4 per cent milk (F.C.M.) for 28 days were as follows: On corn silage, 777.4 lb.; on corn-treated meadow crop silage, 777.9 lb.; and on corn and acetic acid treated meadow crop silage, 776.5 lb.

The carotene and vitamin A contents of the milk produced on the three silages showed little difference, although the meadow crop silages supplied from two to three times as much carotene as the corn silage. This lack of difference may have been due to the shortness of the feeding periods.

P38 The Digestibility of Coarsely Ground and Finely Ground Alfalfa for Dairy Heifers.[1] ERIC W. SWANSON AND A. C. RAGSDALE, Department of Dairy Husbandry, University of Missouri.

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[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1050.

In the course of metabolism and digestibility studies with various feeds, it was noted that dairy heifers nearly ceased ruminating on certain rations containing finely ground roughage. This suggested a study of the effect of suppressed rumination upon digestibility. Alfalfa hay of medium quality, ground or chopped into three different degrees of fineness, was fed. Digestion coefficients were determined with 16-month-old dairy heifers. Rumination was normal on the coarse hay, noticeably depressed on the medium ground hay, and not observed on the finely ground hay. No undesirable effects were observed on any of the rations, and no significant differences were found between the rations in regard to digestion coefficients of total organic matter, total dry matter, or total digestible nutrients. The average T.D.N. was 53.48 ± 1.23 per cent for the finely ground, 52.01 ± 0.14 per cent for the medium ground, and 53.35 ± 0.70 per cent for the coarsely chopped alfalfa. Differences among the rations in the digestion coefficients of ether extract and crude fiber likewise were not significant. The digestion coefficient of nitrogen-free extract for the medium ground hay was significantly lower than for either the finely ground or coarse chopped hay. Protein digestibility also was lower for the medium ground hay than for the other two, but the differences were of doubtful significance. The results of the experiment would indicate that grinding alfalfa hay fine enough seriously to affect rumination does not alter the digestibility. The data suggest that rumination may not be essential to proper digestion of roughage provided it is fine enough not to require remastication.

P39 Drying Requirements, Losses Caused by Drying, and Quality of Forage Obtained in Mow Hay-drying Experiments at Beltsville.

J. B. Shepherd, H. G. Wiseman, and C. G. Melin, Bureau of Dairy Industry; L. G. Schoenleber, Bureau of Plant Industry, Soils and Agricultural Engineering; and W. H. Hosterman, Production and Marketing Administration; U. S. Department of Agriculture.

Seven experimental lots of alfalfa or of mixtures of alfalfa, Ladino clover, and bluegrass, and two lots of a mixture of orchard grass and Ladino clover were dried on barn-sized mow hay finishers. Four lots were dried long and three were dried chopped, using 15 to 16 cu. ft. of unheated air per minute per square foot of floor area. Two lots of long hay, one loose and one field-baled, were dried with different quantities of air and supplemental heat. No rainfall occurred between cutting and storage of any lot. The fan was operated almost continuously during the first 3 or 4 days of drying. The weather during drying was indifferent, and in some cases was poor for drying.

All lots on which leaf determinations were made were either leafy or extra leafy. The drying time was shortest, nutrient losses lowest, and the quality the highest when hay was stored with a low moisture content and dried in medium or shallow depths or dried with supplemental heat. Drying time and nutrient losses increased, and the aroma, green color, and carotene content decreased as the moisture content of the hay increased and as the depth to which it was stored increased. Static pressures increased as the moisture content and the depth of hay increased. Fan use (per ton of dry matter preserved), supplying air at a uniform rate, was lowest with hays having low to moderate moisture contents, with hays stored at medium to deep depths, and with hays dried with supplemental heat; it was highest with hays having high moisture contents, hays stored at shallow depth, and hays dried with unheated air. Electricity consumption (per ton of dry matter preserved) was lowest for hay with low to moderate moisture content and for medium depths of hay, and highest with hay having a high moisture content and with hay stored at deep or very shallow depths.

Samples taken at different depths after drying indicate that the green color and carotene content of the hay is greater at the bottom of the pile, which dries first, than at the top of the pile, which dries last. Carotene determinations made on various dates after drying show that there is a continuous and considerable loss of carotene throughout the storage period from drying to feeding.

The most feed was preserved and the best quality of hay was produced when the hays were dried in a week or less. Losses usually were moderate and the feeding quality very satisfactory when the hay was dried within 10 or 11 days.

Drying the hay down as much as possible in favorable weather without leaf shattering, limiting the quantity of hay to a reasonable depth, and using plenty of air in drying are recommended practices.

The total weight of water in the hay was the principal factor affecting the length of the drying period, the losses from drying, and the quality of the hay.

P40 Grazing Management Studies of Orchard Grass-Ladino Clover.

P. S. Williams, V. G. Sprague, C. B. Knodt, and K. M. Autrey, Department of Dairy Husbandry, Pennsylvania State College, and the U. S. Regional Pasture Laboratory, State College, Pennsylvania.

A study is being conducted to determine the effects of different methods of grazing management upon the maintenance of clover and upon the digestible nutrient production of orchard grass-Ladino clover association. A field of orchard grass-Ladino clover which was established during 1944 was divided in the spring of 1945 into 15 plots approximately 0.7 of an acre in size. Five methods of management were randomized in three replications.

To determine the digestible nutrients produced, the plots were grazed according to the herbage present and the designated grazing scheme for that plot irrespective of the condition of the other replicates of the same treatment. In addition four samples of herbage were harvested with a mower from each plot just before the cattle were turned in for grazing. These samples were used to determine the dry matter yields, chemical composition of the forage, and the botanical composition of the sward.

The digestible nutrient production from grazing of the various plots was determined by calculation of maintenance requirements, gains and losses in body weight, milk production, and barn feed intake of the cows while they grazed each plot, according to the methods recommended by the Joint Pasture Committee.

The mean plot production during 2 years in terms of digestible nutrients obtained from grazing on the basis of the pounds per acre per treatment were as follows:

I Early spring grazing, followed by rotational grazing, 2,930
II Deferred spring grazing, followed by rotational grazing, 4,005
III Early spring grazing, second crop cut for hay or silage and subsequent crops rotationally grazed, 3,460
IV First crop cut for silage, subsequent crops rotationally grazed, 2,896
V Continuous grazing, 3.159

While the mean production from the plots under deferred grazing was somewhat more than from those under other methods of management, a statistical analysis of the data for the first 2 years indicated no significant differences in digestible nutrient production between treatments, between plots, or between years under the conditions of this experiment. Decreases in the clover content of the sward have occurred under certain of the grazing management treatments, and accordingly changes in the relative production of the various plots may be expected in subsequent years.

P41 The Evaluation of Several Grass-Legume Mixtures for Grass Silage and Aftermath Grazing.

C. B. Knodt, V. G. Sprague, P. S. Williams, and K. M. Autrey, Department of Dairy Husbandry, Pennsylvania State College, and the U. S. Regional Pasture Laboratory, State College, Pennsylvania.

One of the most important pasture problems in the Northeastern United States is to provide nutritious herbage during midsummer. Since permanent pastures provide adequate grazing during the spring, the first crop from semi-permanent pasture is most valuable for hay or silage and the aftermath is grazed. In an effort to obtain information on the relative productivity of five associations of grasses and legumes throughout the midsummer months, three replicate plots of approximately 0.3 acre in size of each of the associations were seeded in the spring of 1944. The first crop was harvested for silage when the grass was in the early head stage in the Ladino associations and from the alfalfa plots when the legume was in about the one-tenth bloom stage. The aftermath from all plots was grazed rotationally. The frequency of grazing was determined by the amount of herbage present on an individual plot and its stage of growth, irrespective of the condition of the other replicates or plots of the other associations in the study.

The digestible nutrient production from grazing of the various plots was determined by calculation of maintenance requirements, gains and losses in body weight, milk production, and barn feed intake of the cows while they grazed each plot according to the methods recommended by the Joint Pasture Committee. The following digestible nutrient production was obtained from the various associations during the first 2 years of the experiment (expressed as pounds of digestible nutrients per acre):

	Grass-legume association	*Silage*	*Grazing*	*Total*
I	Orchard grass-Ladino	2055	2439	4494
II	Orchard grass-alfalfa	2201	3212	5413
III	Bromegrass-alfalfa	3022	1730	4752
IV	Bromegrass-Ladino	2260	1826	4086
V	Timothy grass-Ladino	2386	1900	4286

The 2-year averages obtained suggest that the bromegrass-alfalfa combination excelled in production of silage and that the orchard grass-alfalfa and orchard grass-Ladino clover associations provided the greater aftermath grazing. However, a statistical analysis of the data failed to reveal significant differences.

P42 The Influence of Thyroprotein in the Ration of Dairy Cattle.[1]

RALPH P. REECE, New Jersey Agricultural Experiment Station.

Eight dairy cows, past their lactational peaks, received 15 g. of thyroprotein daily for various periods of time (3 to 8.5 months). The average increase in milk production from the week prior to thyroprotein feeding to the highest subsequent weekly production was 24.8 per cent (range 7.9 to 36.2). The fat content of the milk was increased initially in every instance; however, in one case it eventually decreased to a level below that observed prior to thyroprotein feeding.

Two cows (621 and 652) received 15 g. of thyroprotein daily for 10 weeks. Based on weekly averages, milk production of cow 621 increased from 30.7 to 40.0 lb. per day, and that of cow 652 increased from 23.4 to

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[1] Journal Series Paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Husbandry.

31.6 lb. per day. The blood sugar level of the first cow increased from 36 to 53 mg. per cent in the second week of thyroprotein feeding; that of cow 652 increased from 38 to 55 mg. per cent. From the second week to the end of the thyroprotein feeding period, blood sugar levels remained rather constant; however, this did not prevent a decline in milk production.

Thyroprotein was administered to four cows with ketosis. An Ayrshire cow that had calved normally had increased in milk production until the beginning of the third week following calving. After that time milk production declined and a qualitative test on the urine for ketone bodies was positive (4+). Fifteen grams of thyroprotein were fed daily, and milk production, based on weekly averages, increased from 42.4 to 59.6 lb. per day. A Jersey cow in which ketosis had developed to a stage where feed was not being consumed failed to respond to thyroprotein administered orally in a capsule. Another Jersey cow showed symptoms of ketosis in the second week after calving. Milk production declined slightly, feed was not being eaten well, blood sugar level was low (26 mg. per cent), and a qualitative test on the urine for ketone bodies was positive (3+). The daily feeding of 15 g. of thyroprotein resulted in an increase in milk production and in blood sugar level (to 45 mg. per cent), and the qualitative test for urinary ketone bodies became negative. The gradual withdrawal of thyroprotein from the ration resulted in only a slight decrease in milk production and in blood sugar level. A Guernsey cow, 3 days after calving, had a low blood sugar level (20 mg. per cent) and gave a positive qualitative test for urinary ketone bodies. She responded to thyroprotein therapy, and thyroprotein successfully was withdrawn from the ration after a 32-day feeding period.

P43 Some Effects of Feeding Thyroprotein to Dairy Cows. J. W. THOMAS AND L. A. MOORE, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

The effects of feeding thyroprotein to producing dairy cows during the lifetime of the cow are under study at the Agricultural Research Center, Beltsville. Last year it was reported that cows which received thyroprotein and were fed total digestible nutrients according to Morrison's standard showed excessive losses in body weight. Further weight losses were checked by increasing the T.D.N. intake 25 per cent above the Morrison standard. Since then two heifers in their first lactation have received T.D.N. at the rate of 125 per cent of Morrison's standard throughout the thyroprotein-feeding period. Under these conditions no abnormal weight losses were noted and the stimulus to milk production was sustained throughout the entire lactation.

All cows in their second lactation have received T.D.N. at the rate of 125 per cent of the Morrison standard throughout the thyroprotein-feeding period. Some have responded with a sustained increase in milk production while others, after the initial increase, have declined at approximately a normal rate. Most cows responded with an increased butterfat percentage in their milk when thyroprotein was fed.

Heart rates have been observed by means of a stethoscope. Heart rates were increased markedly by feeding the extra T.D.N. along with thyroprotein. Generally the heart rate increased with the increased milk production and decreased when the cow's production decreased. This parallelism also has been noted in cows fed normally. More cases of heart murmurs occurred among the thyroprotein-fed cows than among the other cows. During the second lactation of thyroprotein feeding some of the cows have shown an abnormal sinus arrhythmia, which has been verified by electrocardiograph records.

Eight cows have calved after receiving thyroprotein in their first lactation and one cow has calved after receiving thyroprotein in two lactations. The condition of the cows and all the calves has been normal. The Jersey cows always have conceived after one or two services. Some of the Holstein cows have shown irregular estrus periods and/or have required more than three services in order to conceive. The experiment is being continued to determine the effects of feeding thyroprotein during the lifetime of the cow.

P44 The Effect of Thyroprotein and Feed Intake on the Heart Rate of Dairy Steers. J. F. SYKES, T. R. WRENN, L. A. MOORE, AND J. W. THOMAS, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

The effects of variations in feed intake with and without thyroprotein have been studied in four dairy steers. The data show that increases in feed intake above normal standards increased heart rate and reduction in feed decreased heart rate. Thyroprotein increased heart rate to about the same extent whether the animals were on a high or low level of feed intake, but the absolute heart rates observed were greater on the high level than on the reduced level of intake.

When considered in conjunction with data previously reported on the effect of thyroprotein on milk production, these data suggest that thyroprotein can be effective in increasing milk production only when sufficient extra feed is present to maintain the heart rate above normal levels. Unless feed in excess of accepted standards is given, there may be a gradual depletion of body stores under the influence of thyroprotein until the cow actually may be underfed and milk production be depressed.

P45 Further Observations on the Effects of Feeding Thyroprotein to Dairy Cows. A. H. VANLANDINGHAM, GEORGE HYATT, JR.,

CHARLES E. WEAKLEY, JR., AND H. O. HENDERSON, West Virginia Agricultural Experiment Station.

Previous reports on the feeding of thyroprotein have been made (Jour. Dairy Sci., **27**: 385. 1944; *ibid.*, **29**: 533. 1946).

Thyroprotein (protomone) has been fed to a group of three Jersey and four Holstein cows with one or more lactations completed, beginning the second or third month of lactation and continuing the remainder of the lactation period. Jerseys were fed 15 g. and Holsteins 20 g. of protomone daily. The cows were started on protomone feeding during the winter months and continued through spring to early summer; they were on pasture in season. Protomone was not fed during the dry period. All cows except one Holstein were with calf when protomone feeding was started. Three of the cows, two Jerseys and one Holstein, are approaching the end of the second lactation period on protomone.

All cows calving have dropped normal calves after protomone was fed during the latter part of the previous lactation period. However, there is some indication that cows may be slower to get with calf following the feeding of protomone during the previous lactation. The most noticeable reaction to protomone feeding was the thin condition and loss in body weight after several months of feeding. Increased heart beat and respiration rate, observed at first, seem to approach normal after several months' feeding when the cows were fed in proportion to milk and fat production.

Ascorbic acid in the milk of the Jersey fed protomone was significantly lower than in a similar group not fed protomone. The ascorbic acid in the milk of Holstein cows fed protomone was somewhat lower than in the milk of a control group, but the difference was not significant. Ascorbic acid in the milk of Jersey cows not fed protomone was significantly higher than that of the Holsteins. Ascorbic acid in the milk decreased toward the latter part of the lactation period. Protomone did not affect the ascorbic acid content of blood plasma.

P46 Absorption and Elimination of Thiouracil in Ruminants.

R. E. ELY, K. J. OLSON, AND E. P. REINEKE, Michigan Agricultural Experiment Station.

Absorption and elimination of thiouracil after subcutaneous and oral administration to goats and calves has been studied. Blood plasma thiouracil levels have been determined during continued administration of effective levels of thiouracil to goats and calves by subcutaneous and oral routes.

Maximum blood plasma thiouracil concentrations were reached in 4 to 8 hours after oral administration of thiouracil to goats. The plasma values are approximately proportional to the dosage of thiouracil when administered at the rate of 0.015, 0.03, 0.06, and 0.12 g. per kg. of body weight. The

thiouracil was practically eliminated from the blood plasma in 12 hours at the lower dosage level and in 24 hours at the high dosage level.

Subcutaneous administration of thiouracil to goats gave maximum plasma concentrations in 2 to 4 hours; the compound was eliminated from the plasma quite rapidly. Subcutaneous administrations gave approximately twice the maximum blood plasma thiouracil concentrations as comparable oral dosages. Subcutaneous and oral administration of thiouracil to calves gave similar absorption and elimination curves.

Studies of continued administration of thiouracil to goats and calves indicate that 0.1 g. of thiouracil daily per kg. of body weight administered subcutaneously at 12-hour intervals maintained effective blood plasma thiouracil concentrations. Oral administration of 0.2 g. of thiouracil daily per kg. of body weight at 12-hour intervals also maintained effective blood plasma thiouracil concentrations in goats and calves.

P47 The Influence of Thiouracil on Mammary Lobule-Alveolar Growth in Mice. JOHN P. MIXNER, New Jersey Agricultural Experiment Station.

Thyroidectomy has been observed to decrease the mammary lobule-alveolar growth responses of castrate virgin female mice to injected progesterone and estrone. A group of chemicals related to thiouracil induces the hypothyroid state in animals by inhibiting the synthesis of thyroxine by the thyroid gland. Thus it might be expected that thiouracil also might inhibit mammary responses in mice in a manner similar to that caused by thyroidectomy.

Ovariectomized virgin female mice were used in these experiments and the criteria of response were both the degree of lobule-alveolar growth obtained and the per cent of mice showing mammary lobule-alveolar growth responses. Anterior pituitary extract plus estrone and progesterone plus estrone were given as substances stimulating mammary growth. Thiouracil was given to certain groups of mice as 0.1 per cent of the ration for 2 weeks preceding the experimental period and was continued through the 10 days of the experimental injection period. The animals were ovariectomized immediately preceding the injection period.

Thiouracil decreased markedly both the degree of mammary lobule-alveolar growth and the percentage of mice showing mammary lobule-alveolar growth responses in the group injected with progesterone plus estrone and that injected with anterior pituitary extract plus estrone.

It may be concluded that thiouracil acts in a manner similar to thyroidectomy in decreasing the responsiveness of the mammary gland to exogenous mammary growth stimulating substances.

P48 Factors Influencing the Male Hormone Content of Cow Manure.[1] C. W. TURNER, Missouri Agricultural Experiment Station.

In continuing the study of the androgenic (male) hormone content of dairy cattle manure during the past year, samples were collected from 30 dairy cows in various stages of lactation. Of these cows 18 were Holsteins, 8 were Jerseys, and 4 were Guernseys. The manure was dried in an electric oven at 45° C. and ground in a small hammer mill. The hormone content of the sample of manure was assayed by feeding it, as 10 per cent of the ration, to about 20 White Rock chicks for 28 days. The combs then were removed and weighed. The average weight of combs of the two sexes was divided by two.

For comparison with the experimental results, a group of similar control chicks has been run to determine the extent of seasonal variation in the normal chick-comb weight. Evidence of seasonal variation is appearing. The average normal comb weight during the 7-month period from September to March was 70.9 mg. Chicks fed 20 mg. methyl testosterone per kilo feed had combs averaging 319.3 mg. during the same 7-month period. It is planned to correct all data for seasonal variation. However, the results presented are uncorrected.

The average comb weight of the chicks fed 11 samples of manure from lactating Jersey cows was 189.62 mg., with a variation from 123 to 280. Seven samples from Guernsey cows averaged 160.4 mg., with a variation from 100 to 239 mg. The 32 samples of manure from lactating Holstein cows averaged 145.3 mg., varying from 71 to 233 mg.

The content of male hormone seems to be excreted in highest amount a few days before parturition and during the first month of lactation. After the first month the hormone content is reduced but remains fairly constant for much of the lactation period. Breeding lactating cows does not appear to cause any marked effect. If cows go dry for several months before the next parturition, the hormone content tends to be lower until the preparturient rise occurs.

Considering the many variable factors observed, the data available at this time can be considered only suggestive. Much further work is required.

P49 Progress Report in the Study of Certain Goitrogens.[2] G. W. PIPES AND C. W. TURNER, Missouri Agricultural Experiment Station.

In view of the possibilities of the more active goitrogens as fattening agents for dairy calves and other livestock, a study has been made of the

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1049.

[2] Contributions from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1048.

concentrations of thiouracil, 6-n-propyl thiouracil, and thiobarbital (5,5-diethyl-2-thiobarbituric acid) in the blood and tissues of several species of domestic animals during oral administration.

The same general pattern of rapid absorption from the digestive tract into the blood stream and speedy elimination from the blood and tissues has been observed in the domestic fowl, rabbit, goat, sheep, and calf. Significantly higher blood levels were produced and maintained in the blood stream of the fowl and the goat by thiobarbital than by any of the other goitrogens which were investigated.

Two 125-lb. Holstein calves were given 2 g. of thiouracil by capsule at 12-hour intervals. Thiouracil concentrations in the blood were determined at intervals during a 24-hour period. It was found that a uniform level could be maintained in the blood.

Thiouracil and thiobarbital concentrations in the tissues of animals receiving these goitrogens have been determined by a biological assay based on hypertrophy of the rat thyroid. Meat from animals receiving goitrogens, when fed to rats as 95 per cent of the diet, produced an almost imperceptible increase in thyroid size. The goitrogenicity of all tissues studied was less than that produced by the addition of 5 mg. of thiouracil per 100 mg. of normal tissue.

P50 The Effect of the Interval between Washing of the Udder and Attachment of Milking Machines upon the Bacterial Flora and Milk Production of Dairy Cows. C. B. Knodt, J. J. Reid, P. S. Williams, and E. M. Kesler, Department of Dairy Husbandry and the Department of Bacteriology, Pennsylvania State College.

During recent years considerable emphasis has been placed upon the importance of managed milking of dairy cows. The newer methods of milking not only save time but also are believed to increase milk production and to decrease the incidence of mastitis. Many dairymen do not attach milking machines during the recommended time after washing the cow's udder because of milking parlor arrangements or for other reasons. Accordingly, studies were conducted to determine what effect delay in attaching milking machines after washing the cow's udder might have upon milk production and the bacterial flora of the udder. All cows in the experiments were milked twice daily and machine stripped. The udders were washed carefully with water at 125 to 130° F. for approximately 15 seconds each. Two large streams of milk were removed from each teat following the washing. Use of the strip cup gave no evidence of any difference in the response of any of the groups. The cows were fed and managed similarly.

Thirty cows, with an average daily production of 51.19 lb., were divided into five similar groups on the basis of milk and milk fat production, stage of gestation, and stage of lactation. The milking machines were attached

2, 4, 6, 8, and 10 minutes after the washing was started. Statistical analysis of the milk production of the groups during the 35 days of this trial demonstrated no significant difference between treatments. The apparent change in bacterial flora of the udder was characterized by the appearance of β-hemolytic streptococci and long chain alpha and gamma streptococci in those groups where milking was delayed more than 4 minutes after the beginning of washing.

Because of the need for cows on certain pasture management projects, only 12 of the cows (four in each interval of 2, 6, and 10 minutes) were continued for an additional 35 days. Statistical analysis of the data covering 70 days' milk production showed no significant differences resulting from the various treatments and no changes in the bacterial flora of the udders which could be related to treatments.

In another experiment, 20 cows, with an average daily milk production of 39.6 lb., were divided into four comparable groups on the same basis as in the previous trials. The time intervals used were 2, 5, 10, and 20 minutes. As in the previous experiments, no statistically significant differences could be found in milk production during the 90-day experimental period. While some changes were observed in the concentration of leucocytes of the milk of various cows, these were believed to be related to stage of lactation. No changes were observed in the bacterial flora of the udders of the cows in the third trial. Under the conditions of this experiment, there was no significant effect upon milk production of time intervals up to 20 minutes between the beginning of washing a cow's udder and attachment of the machines.

While in the first experiment it appeared that definite changes occurred in the udder bacterial flora by delaying milking longer than 4 minutes after washing the cows' udders, later experiments did not agree with this observation.

P51 The Riboflavin Content of Cow's Colostrum. T. S. SUTTON AND HAROLD E. KAESER, Ohio State University and Ohio Agricultural Experiment Station.

The content and total output of riboflavin in the colostrum and subsequent milk of 35 cows was determined for the first ten consecutive and the twentieth milkings. Five dairy breeds were included in the study. The calves were not allowed to nurse and the total production was milked out, weighed, and sampled for analysis. Riboflavin was determined fluorometrically.

The average riboflavin content in milligrams per liter was found to be: 6.15, 3.66, 2.44, 2.25, 2.20, 2.10, 1.98, 2.01, 1.96, 1.95, and 1.83 for the first ten and the twentieth milkings, respectively. The total output of riboflavin was: 33.1, 12.6, 12.2, 14.8, 14.5, 15.1, 14.5, 13.7, 14.6, 14.3, and 16.0 mg., respectively, for the first ten and the twentieth milkings.

Considerable individual variation in riboflavin composition was found. For example, the potency of the first milking varied from a high of 11.5 mg./l. to a low of 3.9 mg./l. Breed differences in the riboflavin content of colostrum and milk also are indicated.

The production of riboflavin deficiency in very young calves by Illinois investigators (Jour. Nutr., **33**: 263–270. 1947.) gives emphasis to the importance of colostrum and milk as a source of riboflavin in the nutrition of the young calf.

P52 Some Possible Relationships between Management, Fore-stomach Contents and Diarrhea in the Young Dairy Calf. W. D. POUNDEN AND J. W. HIBBS, Ohio State University.

The color of the feces of 24 new-born calves was brown or yellow for approximately the first week, depending somewhat on the duration of colostrum feeding or nursing period. A change to gray shades of yellow or white followed. At approximately 2 weeks of age another change to darker shades of gray brown or green occurred, apparently influenced by the type and quantity of dry feeds consumed. Although few calves had diarrhea during the first and third stages, all but one had periods of abnormally liquid feces for a few days during the second stage. Practically all recovered without the assistance of drugs.

The materials removable from the fore-stomachs by use of stomach tubes (inside diameter 7 mm.) increased considerably for a day or so in nine of ten Jersey calves between 7 and 10 days of age, and in eight Holstein calves between 6 and 9 days and again in six of them between 10 and 13 days of age. The quantity that could be removed depended on the volume present and the consistency. These ages are within the same age period during which diarrhea attacks were prevalent. In ten instances (among 19 calves) increases in rumen liquids coincided within a day with the appearance of diarrhea. Milk or curd was present in the rumen contents of eight of twelve 1-day old calves, but was observed on only 4 of 23 occasions at the time of increased removable materials. Diarrhea accompanied the latter and the calves were depressed. A reading of pH 4.43 was obtained on one of these curdy samples in contrast to pH 6.7, the average obtained for 43 samples from nine of the calves up to 10 days of age.

Rumen flora and fauna judged to be normal on the basis of the presence of organisms similar to those observed in cows developed in but five of fifteen calves within 30 days of birth and still were absent from the rumens of two of seventeen calves at 90 days of age. The two calves that suffered most from indigestion when placed exclusively on dry feed were classified as not having normal flora and fauna by 5 weeks of age. Five calves provided with organisms from mature animals by transfer of rumen contents or cuds were among those that progressed the most satisfactorily.

The limited nature of the observations precludes definite conclusions. However, indications are that it may be advisable to endeavor to limit the duration of the period when calves have the second type of feces. Perhaps this might be accomplished by extended colostrum feeding, coupled with early provision of suitable dry feeds along with rumen flora and fauna.

P53 The Effect of Feeding Various Levels of Vitamin A on the Depletion Time of Dairy Calves. W. C. Jacobson, H. T. Converse, and L. A. Moore, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

In order to study the time required for the depletion of the vitamin A stores of calves reared under natural conditions, two pregnant cows were turned on pasture early in the spring about 3 weeks before calving. The two heifer calves which they dropped were permitted to run with their dams on pasture from birth to 90 days of age. Thus, these two calves could obtain whatever quantity of summer whole milk they desired and, in addition, any green grass they would consume.

Male calves receiving colostrum milk for 3 days and a limited quantity of whole milk to 60 days of age, along with a grain mixture and U.S. no. 2 alfalfa hay, also were placed on experiment. The whole milk fed was from cows receiving winter rations. One calf out of each pair of calves received, in addition, various quantities of vitamin A in the form of cod-liver oil for various periods of time.

At 90 days of age all calves were placed on a vitamin A-deficient ration to determine how long it would take to deplete their stores of vitamin A. Stores were considered to be depleted when the vitamin A of the blood plasma decreased to a level of 4 μg. or less per 100 ml. of plasma.

The two calves which ran on pasture with their dams required up to 4 months for depletion, whereas calves which received no supplemental vitamin A and were reared according to present day methods of limited whole milk feeding required less than 30 days, in most cases, for the depletion period. Where 50,000 I.U. of vitamin A per day were fed for various periods of time, the depletion time was in proportion to the quantity of vitamin A fed.

P54 The Antirachitic Properties of Field-cured Hay, Barn-dried Hay, and Wilted Grass Silage when Fed to Calves. L. A. Moore and J. W. Thomas, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

Exposure of hay to the rays of the sun during the curing process increases its vitamin D potency. In making barn-dried hay and wilted grass silage, the length of time the crop is exposed to the sun's rays is considerably less than in making field-cured hay. Thus it might be reasoned that barn-dried hay and wilted silage contain less vitamin D than field-cured hay.

Roughage was put up as field-cured hay, barn-dried hay, and wilted silage, and each kind of roughage was fed to six dairy calves to study its antirachitic effect.

The ash analysis of the first 10 per cent of the epiphyseal ends of the seventh and eighth ribs did not reveal any particular differences between the calves fed the different roughages. In all three groups of calves, the rib ends of those that were fed at the lower roughage levels were lower in ash. There was some indication that the calves fed the barn-dried hay and the wilted silage showed more stiffness than the calves fed the field-cured hay. Calcium, phosphorus, and phosphatase determinations of the blood did not reveal any difference between the three groups.

Wilted silage made from the 1946 crop was fed to two calves, beginning at birth. The only other source of vitamin D which the calves received was from a limited quantity of whole milk fed to 60 days of age. One calf now is 5 and the other 7 months old, and there is no physical indication of developing rickets. The blood picture in both calves also has remained normal.

While further studies must be made, these results would indicate that where barn-dried hay and wilted grass silage are fed at the usual levels of roughage feeding (*i.e.*, 2 to 3 lb. of hay or its equivalent per 100 lb. of body weight), sufficient vitamin D is present to prevent rickets in growing calves.

P55 Changes in the Cell Volume and in the Concentration of Several Inorganic Constituents in the Blood of the Dairy Calf during Its Early Postnatal Development. G. H. WISE, M. J. CALDWELL, D. B. PARRISH, AND J. S. HUGHES, Kansas Agricultural Experiment Station.

Changes in cell volume, hemoglobin, calcium, magnesium, sodium, and potassium of whole blood and in calcium, magnesium, sodium, potassium, and inorganic phosphorus of blood serum from 11 individual dairy calves were observed during the normal dietary transitional stages from birth to effective hay ingestion. Blood samples were collected immediately after birth, daily during the colostral period, weekly from the first to the sixth week, and biweekly thereafter to 10 weeks of age.

Cell volume and hemoglobin decreased from birth to approximately 3 weeks of age but gradually increased thereafter, suggesting a response to hay ingestion. Though sodium revealed no striking change at any stage, the concentration in whole blood decreased from birth to 2 weeks of age, followed by a gradual increase. The significance of these changes is rather obscure. Potassium of whole blood declined throughout the first 8 weeks of postnatal life; that of the serum remained relatively uniform. Inorganic phosphorus of the blood serum increased during the first week but subsequently remained at a constant level. Though calcium of the whole blood and of the serum decreased after the period of colostrum consumption, the change is of doubtful significance. The magnesium concentrations were uniform throughout. The changes in the concentrations of these various constituents of the blood apparently were not affected to any marked degree by dietary changes.

P56 The Value of Distillers Dried Solubles and Dried Grains with Solubles in the Ration of Dairy Calves.[1]

J. R. SCHABINGER AND C. B. KNODT, Department of Dairy Husbandry, Pennsylvania State College.

A total of 48 calves, purchased in Central Pennsylvania, were divided into six comparable groups on the basis of breed, sex, body weight, height at the withers, and circumference of the chest at the beginning of the trial. Because of the difficulties encountered in obtaining sufficient calves, two male and two female calves of each of the Holstein and Guernsey breeds were used in each group.

The following rations (in pounds) were utilized:

Group I (Basal) —(crushed oats, 650; corn meal, 595; wheat bran, 165; soybean oil meal, 250; linseed oil meal, 120; dried whey, 100; dried brewer's yeast, 60.)
Group II —(crushed oats, 650; corn meal, 595; wheat bran, 175; soybean oil meal, 250; linseed oil meal, 120; distillers dried solubles, 150.)
Group III—(crushed oats, 650; corn meal, 595; wheat bran, 145; soybean oil meal, 250; distillers dried solubles, 300.)
Group IV —(crushed oats, 650; corn meal, 595; wheat bran, 245; soybean oil meal, 250; distillers dried solubles, 150; redfish meal, 50.)
Group V —(crushed oats, 650; corn meal, 595; wheat bran, 175; soybean oil meal, 250; linseed oil meal, 120; distillers dried grains with solubles, 150.)
Group VI —(crushed oats, 650; corn meal, 595; wheat bran, 145; soybean oil meal, 250; distillers dried grains with solubles, 300.)

In addition, all rations contained steamed bone meal, 25 lb.; ground limestone, 15 lb.; salt, 10 lb.; vitamin A oil (containing 3,330 U.S.P. units per g.), 9 lb.; and irradiated yeast, 1 lb.

All calves were fed 300 lb. of whole milk during the first 45 days on the trial. Mixed hay (timothy and Ladino clover) was before the calves at all times until they were 2 months of age, after which they were fed a good quality alfalfa hay *ad libitum* throughout the remaining 6-month period.

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[1] This work was supported in part by Schenley Distillers Corporation, N. Y., N. Y., and by the Flory Milling Company, Bangor, Pennsylvania.

During the course of the trial the following losses, due to pneumonia and enteritis, were incurred: Group I, 1 Holstein female; Group II, none; Group III, 1 Guernsey female, 2 Guernsey males; Group IV, 1 Guernsey female, 2 Guernsey males; Group V, 1 Guernsey female, 1 Holstein male; and Group VI, 1 Guernsey female.

The following mean daily gains in body weights, for the calves surviving the trial, were observed during the 6-month feeding period (per cent = per cent normal, Mo. Agr. Expt. Sta. Bul. 336.): Group I, 1.42 lb. (102.3 per cent); Group II, 1.46 lb. (109.3 per cent); Group III. 1.48 lb. (98.8 per cent); Group IV, 1.42 lb. (93.7 per cent); Group V, 1.41 lb. (102.1 per cent); and Group VI, 1.50 lb. (104.6 per cent). The mean daily growth rates in terms of height of the withers were as follows (per cent = per cent normal, Mo. Agr. Expt. Sta. Bul. 336.): Group I, 0.135 cm. (91.3 per cent); Group II, 0.131 cm. (85.8 per cent); Group III, 0.136 cm. (91.0 per cent); Group IV, 0.132 cm. (88.4 per cent); Group V, 0.124 cm. (85.7 per cent); and Group VI, 0.133 cm. (90.1 per cent). The mean daily growths, measured in terms of circumference of the chest, were as follows: Group I, 0.23 cm.; Group II, 0.25 cm.; Group III, 0.22 cm.; Group IV, 0.23 cm.; Group V, 0.23 cm; and Group VI, 0.25 cm. Careful observation of the data reveals that the variation between calves within groups was as great as the variation between groups when gains in body weight, height at the withers, and circumference of the chest are considered as criteria of growth. Accordingly, it appears under the conditions of this experiment that distillers dried solubles and distillers dried grains with solubles may replace satisfactory linseed meal, dried brewer's yeast, and dried whey in the rations of dairy calves.

P57 The Utilization of β-Carotene, Vitamin A Alcohol, and the Natural Ester of Vitamin A by Holstein Heifers.[1]

R. H. Ross, C. B. Knodt, and N. B. Guerrant, Departments of Dairy Husbandry, and Agricultural and Biological Chemistry, Pennsylvania State College.

Six Holstein heifers between the ages of 12 and 15 months were used in an experiment to determine the relative efficiency of utilization of β-carotene, the natural ester of vitamin A, and vitamin A alcohol. The heifers were fed throughout the trials oat straw *ad libitum* and 10 lb. per day of a concentrate mixture containing 200 lb. of oat feed, 200 lb. ground oats, 100 lb. peanut bran, 125 lb. soybean oil meal, 10 lb. steamed bone meal, 7 lb. salt, and 0.5 lb. irradiated yeast. When the blood plasma vitamin A levels were reduced to 6–8 μg. per 100 ml., the heifers were divided into three similar pairs on the basis of body weight, age, and blood plasma vitamin A concentrations.

[1] This work was supported in part by The Borden Company, N. Y., N. Y.

The three sources of vitamin A then were administered in capsule form for 20 days at the rate of 100 U.S.P. units per kg. of body weight per day. After each of the first two periods of administration, the heifers again were depleted and the supplementation rotated so that after three feeding periods all six heifers had received the three sources of vitamin A for a 20-day period.

Daily blood samples were taken during the periods of administration except during the last half of the first trial, when they were taken every other day. In analyzing the data representing the blood plasma vitamin A determinations for the three treatments by an analysis of variance, a highly significant difference was found between the treatments and between days of supplementation. The difference between heifers approached significance. It was determined that no significant difference existed in the blood plasma vitamin A levels of heifers during the administration of vitamin A alcohol or the natural ester of vitamin A. However, both produced a higher level (highly significant) of blood plasma vitamin A than did β-carotene. The following mean concentrations of blood plasma vitamin A (μg. per 100 ml.) were found during the feeding periods: β-carotene feeding, 6.95; vitamin A alcohol feeding, 10.77; and the natural ester of vitamin A feeding, 10.83 [t (0.01) = 2.846].

In an analysis of variance of the determinations of blood plasma carotene of the heifers on the three treatments, a highly significant difference was found between treatments and between days on supplementation, but no significant difference was found between heifers. The following mean concentrations of blood plasma carotene (μg. per 100 ml.) were determined during the feeding periods: β-carotene administration, 67.42; vitamin A alcohol administration, 43.59; and the natural ester of vitamin A administration, 49.28 [t (0.01) = 19.05]. On the basis of the least significant mean difference required for significance, it was found that feeding carotene increased the blood plasma concentration of carotene above (highly significant) that of the heifers receiving vitamin A alcohol and the natural ester of vitamin A. There was no significant difference in the blood plasma concentrations of carotene when vitamin A alcohol or the natural ester of vitamin A was fed.

P58 Soybean Oil-filled Milks for Feeding Young Dairy Calves. N. L. Jacobson and C. Y. Cannon, Iowa State College.

Observations were made of the growth and general health of young dairy calves fed various reconstituted milks as compared to whole milk. Five calves were placed on each experimental ration. The calves were fed at the rate of 10 lb. of milk per 100 lb. liveweight. All calves were allowed to remain with their dams for 3 days, after which time they were placed on the experimental rations for 8 weeks. Each calf received 6 g. of sulfathalidine per day per 100 lb. liveweight for 10 days starting at birth. Daily supplements of vitamins A and D and a mineral mixture were given to all calves. All calves were muzzled and received no hay or grain.

One group of experimental animals was fed whole milk containing approximately 3 per cent butterfat. Another group was fed a reconstituted skim milk made by mixing one part of dried skim milk with nine parts of water. Five other groups were fed reconstituted skim milk plus various oils as follows: (a) butter oil, 3 per cent; (b) expeller process soybean oil, 2 per cent; (c) expeller process soybean oil, 3 per cent; (d) expeller process soybean oil, 3 per cent, plus a vitamin supplement including thiamine, riboflavin, calcium pantothenate, pyridoxine, nicotinamide, ascorbic acid, and alpha tocopherol; and (e) hydrogenated soybean oil, 3 per cent. All reconstituted milks were homogenized at 3,000 lb. pressure.

The group receiving 2 per cent soybean oil exceeded the skim milk group in growth, but calves in the soybean oil group scoured frequently. The calves receiving 3 per cent expeller soybean oil scoured badly and were very unthrifty. Three animals in this group died. Supplementation of the milk containing 3 per cent expeller soybean oil with B-complex vitamins, ascorbic acid, and alpha tocopherol neither improved the growth of the calves nor reduced the incidence of scours.

The calves in the group fed 3 per cent hydrogenated soybean oil grew well and were in excellent condition at the end of the experimntal period. There were no significant differences in growth or general appearance between the group fed butter oil and the group which received hydrogenated soybean oil. The calves of both of these groups compared favorably with those fed whole milk.

P59 Placental Transfer and Colostral Storage of Vitamin D in the Bovine.[1] H. D. Eaton, A. A. Spielman, J. K. Loosli, and K. L. Turk, Cornell University.

A study was made during the late winter feeding period and the late pasture season of the effect of a daily supplementary feeding of 100,000 I.U. of vitamin D in the form of irradiated yeast for 8 weeks prepartum on the vitamin D levels in the maternal blood plasma, newborn blood plasma and liver, and in the colostrum.

Two groups of four cows each were started on experiment in March, 1946, and two groups of four cows each in late July and August, 1946. One group at each season served as controls and the other received the daily vitamin D supplement. The groups on experiment during March and April were fed timothy hay, corn silage, and grain, and were allowed outside of the barn daily for approximately 1 hour. The groups on experiment in late July and August were on pasture except at two daily feeding periods,

[1] Supported in part by a grant from Standard Brands, Inc.

and received timothy hay, corn silage, and grain in addition to the pasture. Maternal blood samples were drawn at 8 weeks prepartum before starting vitamin D supplementation and at 2 weeks prepartum. Those calves used in determining blood plasma and liver vitamin D were sacrificed within 3 hours after birth without having received colostrum. Colostrum vitamin D was determined on a composite sample representing the first six milkings postpartum, the first milking being the next regular herd milking after parturition.

Maternal blood plasma vitamin D values, representing three cows per group, increased to a higher level in the supplemented groups than in the controls; however, the difference was not significant. The values for the late pasture season were significantly higher than for the late winter feeding season.

Newborn blood plasma and liver vitamin D values, representing three calves per group, showed no consistent differences between those calves from supplemented dams as compared to those from control dams. Both blood plasma and liver vitamin D levels were higher in the late pasture season than in the late winter period. These seasonal differences were not significant.

Colostrum vitamin D values, representing four cows per group and expressed as I.U. of vitamin D per gram of colostrum fat and I.U. of vitamin D per pound of colostrum, were significantly higher in the supplemented groups than in the controls. Total I.U. of vitamin D in the first six postpartum milkings was on the average higher in the supplemented group; however, this difference was not significant.

These data, although limited in number, suggest that the prepartal diet does influence maternal blood plasma levels of vitamin D and the colostrum content of vitamin D. Seasonal differences are apparent. The negative results in increasing the newborn blood plasma and liver vitamin D content by prepartal vitamin D supplementation are not conclusive but are indicative of the need of higher levels of vitamin D supplementation.

P60 The Effect of Protein Level on the Nitrogen Metabolism and Gains in Weight of Growing Holstein Calves. G. P. LOFGREEN AND J. K. LOOSLI, Laboratory of Animal Nutrition, Cornell University.

Two groups of purebred Holstein calves were raised to 150 lb. by a whole milk-dry starter method. From a weight of 150 to 350 lb., one group received a ration furnishing gradually decreasing amounts of protein beginning at 1.10 lb. and decreasing to 0.93 lb. of digestible crude protein per day. The second group received a ration supplying the same amounts of total digestible nutrients while the protein increased from 0.46 to 0.78 lb. per day. There was no difference in the average daily gain of the two groups of heifers, both groups gaining approximately 1.2 lb. per day for the entire period. The average age of the calves at 350 lb. was 7.5 months, the rate of growth being approximately 70 per cent of the Morrison standard for growing Holstein heifers. The slow growth of both groups suggests that the T.D.N. allowance of the Morrison feeding standard for growth of dairy heifers may be inadequate for normal growth at these weights.

Nitrogen balances were determined on all heifers at body weight intervals of 50 lb. The heifers on the high protein level retained significantly larger amounts of nitrogen between 150 and 250 lb. than those on the lower level. This suggests a greater storage of protein in the one group even though there was no difference in gains.

Using male calves between 150 and 200 lb., nitrogen balances were determined on rations furnishing approximately 0.11, 0.27, 0.42, 0.46, 0.53, 0.64, and 1.10 lb. of digestible crude protein daily. The nitrogen retained increased as the protein in the ration increased, although the percentage of absorbed nitrogen retained was significantly less at the highest level.

Two groups of yearling heifers averaging about 600 lb. were placed on two protein levels. One group was fed an average of 1 lb. of digestible crude protein per day at the beginning and 1.2 lb. at 1,000 lb. body weight. The second group was fed the same T.D.N. level, with the protein decreasing from 0.8 lb. at the beginning to 0.61 lb. at 1,000 lb. Preliminary results on these heifers indicate that good growth might be obtained on considerably less protein than recommended by the Morrison feeding standard for these weights.

EXTENSION SECTION

Canadian Registration and Production Policies. G. E. Raithby, Ontario Agricultural College.

The Dairy Service Cooperative in Ohio. R. R. Starbuck, Ohio State University.

In Ohio the Dairy Service Cooperative is organized and incorporated under the cooperative laws of the state. Fees for incorporation, membership, and services are nominal. Membership is composed of herd owners who are interested in improving their own efficiency, as well as that of their herds, through planned management.

Dairy Service Cooperatives are so organized that major producer activity in a given area (usually a county) may be more easily directed on a united front. They have proved an excellent vehicle through which common objectives may be accomplished and may serve as instruments for making intelligent adjustments when local, national, or international demands for dairy products change to lower or higher levels. Sixty-three of the eighty-eight counties in Ohio are organized on this basis.

Activity of the cooperative is directed by means of a board or central committee. Many, if not all, of the directors serve on subcommittees, which include: Production Testing (both D.H.I. and O.S. testing), Breeding, Breed Promotion and 4–H Club, Disease Eradication, Sales, and Information.

Successful operation of the Dairy Service Cooperative depends on a continuous educational program through county agent activity, vocational agriculture teachers, dairy husbandry specialists, board members, breeding technicians, and testing supervisors. Just as important, and perhaps even more important, is a cooperative membership that recognizes the need for improvement in dairy cattle and management practices.

A Method for Estimating the Yield of Pastures on Farms in D.H.I.A.

R. E. HODGSON AND J. B. SHEPHERD, Division of Nutrition and Physiology, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

A method which is suitable for use by D.H.I.A. testers for estimating the yield of pastures on dairy farms is presented. The method is a modification of the technique reported by the Committee on Pasture Investigations Technique[1] for determining the yield of experimental pastures.

Most of the data needed for calculating the yields can be obtained from the D.H.I.A. records which the tester gets on one day in each month or testing period. The tester also must have data showing the acreage of pasture, the number of cows and heifers by age groups maintained on pasture, and the number of days in the month or testing period that each animal is on pasture. A suitable form has been developed for the use of the tester or herd owner in recording these additional data.

The yield of pasture for a given test period is calculated by figuring the total nutrient requirements of the animals maintained on pasture and by deducting from this figure the total amount of nutrients fed in the barn. The requirements of the animals are based on the average breed weights for the different age classes and on the nutrient requirements for maintenance and production as proposed by the National Research Council Subcommittee on Dairy Cattle Nutrition.[2] These requirements allow enough nutrients for the maintenance of cows 4 years old and over; for maintenance and 0.25 to 0.4 lb. growth daily for 3- to 4-year-old cows; for maintenance and 0.5 to 0.8 lb. growth daily by 2- to 3-year-old heifers; and for maintenance and 1.0 to 1.6 lb growth by 1- to 2-year-old heifers. For determining the nutrients in the supplementary feed given, grain is considered to contain 75, hay 50, and silage 18 per cent T.D.N. The difference between the total nutrients required by the herd and the nutrients consumed from supplementary feed gives the total digestible nutrients the cows obtained from pasture, which, when divided by the number of acres, shows the yield per acre for the period under consideration. Dividing this amount by 16 gives the number of standard cow-days per acre. The sum of the yield data for all periods gives the total seasonal yield of the pasture on the farm. A form suitable for making the few necessary calculations to determine the pasture yield is presented.

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[1] Preliminary report on pasture investigations technique. Joint Committee of American Society of Agronomy, American Dairy Science Association, American Society of Animal Production. Jour. Dairy Sci., 26(4): 353–369. 1943.

[2] Recommended nutrient allowances of domestic animals. III. Recommended nutrient allowances for dairy cattle. A report of the Committee on Animal Nutrition, National Research Council, August, 1945.

The authors have compared this so-called D.H.I.A. method of calculating the yield of pasture with the technical method referred to above, using an experimental pasture at the Beltsville Research Center. With the technical method, the actual liveweights and daily weights of milk production and feed consumption of individual cows were used to calculate the nutrients required for maintenance, gain or loss in weight, and milk production, and to determine the amount of nutrients provided in supplementary feed. The pasture yields as determined by the two methods are shown in the table.

Pasture yields as calculated by two different methods

Method used	Yield of total digestible nutrients in:							
	April	May	June	July	Aug.	Sept.	Oct.	Total
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
D.H.I.A.	301	920	541	360	182	160	174	2,638
Technical	174	1,110	427	431	186	180	180	2,688
Ratio $\frac{\text{D.H.I.A.}}{\text{Technical}}$	1.73	0.83	1.27	0.84	0.98	0.89	0.97	0.98

There was some variation from month to month but for the season as a whole the two methods gave about the same results. Further comparisons with other experimental pastures are being made. The Extension Service of the University of Maryland is cooperating in testing this method under farm conditions in D.H.I.A. herds.

This D.H.I.A. method offers possibilities for a simple easy method of estimating the yield of dairy pastures by using data obtained with grazing animals. It is more specific and more useful than existing ways of estimating and shows effectively the variation in yield of pasture from month to month during the season.

Application of the D.H.I.A. Method of Measuring Pasture Yield.

FLOYD J. ARNOLD AND R. E. SHEAFFER, University of Maryland.

During the pasture season of 1946, a Maryland D.H.I.A. supervisor, R. E. Sheaffer, made pasture yield studies on six of his members' farms. The method used in making this study was the one recommended by Hodgson and

Shepherd for use on farms in Dairy Herd Improvement Associations. Practically all of the work required to make the study was done by Mr. Sheaffer at the time of his regular monthly visit to each farm. In addition to information regularly compiled, pasture information was tabulated on two special forms. The forms were the Pasture Grazing Record and the Pasture Yield Summary.

On the six farms selected, opportunity was provided for studying different types of pastures and the result of different pasture management practices. Both interesting and valuable facts were revealed. The accompanying table shows the yield of digestible nutrients per acre by months on the six different farms.

Total digestible nutrients per acre by months

Farm	*April*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Total*
1	655	828	485	711	594	362	447	439	4522
2	462	867	611	464	426	346	288	171	3640
3	189	869	439	441	378	352	618		3286
4	328	630	255	538	295	451	168	35	2700
5	3	179	205	177	125	186	158	77	1110
6	20	98	107	179	207	157	56	63	887

The following facts concerning the pasture programs on the different farms serve to explain the difference in the yield of nutrients per acre:

Farm 1—There is no permanent pasture land on this farm. Ladino clover and orchard grass, seeded together, is used for pasture. The fields are fertilized regularly and rotational grazing is practiced.

Farm 2—Orchard grass and Ladino clover furnish about three-fourths of the total pasturage on this farm. The remaining one-fourth is permanent blue grass pasture. The pastures are fertilized but no rotational grazing is practiced.

Farm 3—About half of the time the cows on this farm are kept on permanent blue grass pasture and the rest of the time they are on orchard grass. The permanent pastures are fertilized occasionally and the orchard grass is grown in the regular rotation of crops.

Farm 4—Ladino clover and orchard grass furnish most of the pasturage on this farm. In addition there is some woodland pasture where the cows are kept when the Ladino clover and orchard grass is being rested. On the Ladino clover and orchard grass pastures approximately 300 lb. of superphosphate per acre is used annually.

Farm 5—On this farm only permanent blue grass pasture is available. No attempt has been made to increase the pasture yield by use of fertilizer, reseeding, or other pasture improvement practices.

Farm 6—Permanent blue grass pasture is the mainstay on this farm. In the middle of the summer and early fall, however, the second growth clover in the hay fields is grazed.

Selection and Repeatability of Sires Used in Artificial Insemination.

RAY ALBRECTSEN, Cornell University.

The artificial insemination program in New York has progressed to the point of making it possible to get an indication of the repeatability of the proved sires used. Seven proved Holstein sires used by New York State Artificial Breeders Cooperative, Inc., have been re-proved through their daughters in artificial insemination. Each of these sires has 14 or more comparisons in his re-proving; five have maintained or increased production in artificial service as measured by their daughters' records. The data for these bulls is summarized as follows:

		Milk production (lb.)	Fat test (%)	Fat production (lb.)
7 Holstein sires, natural service	183 Daus. Av.	12,800	3.5	451
	Dams Av.	11,610	3.5	408
	Diff.	+ 1,190		+ 43
No. of daughters equal to or better than their dams		126	110	130
7 Holstein sires, artificial service	183 Daus. Av.	12,011	3.6	429
	Dams Av.	11,991	3.5	422
	Diff.	+ 20	+ 0.1	+ 7
No. of daughters equal to or better than their dams		98	109	96

Many factors entering into this summary need to be considered, such as: (a) Variable environment of artificial heifers (76 different herds involved); (b) higher proportion of high producing mates (400 lb. or more fat, Natural—53 per cent, Artificial—64 per cent); (c) quality of previous bulls; and (d) only records actually reported on 718 reports are used.

Of the 183 dams bred artificially to the sires, 114 had average production over 400 lb. of fat, 29 over 500, and 3 over 600 lb. The 183 artificial progeny of these seven sires made records distributed as follows: 124 over 400 lb. butterfat, 31 over 500, 9 over 600, and 1 over 700 lb.

Sires selected for use in artificial insemination as young sires and later proved offer an opportunity to evaluate selection methods under a very severe set of conditions. Five such sires, with 13 or more comparisons, have been proved exclusively through "artificial" daughters. Of these, one has been discarded on the basis of the production of his daughters, which is lower on the average than their dams. All of the bulls were selected on the basis of a herd breeding analysis; the four satisfactory bulls had daughters averaging over 400 lb. fat.

The over-all performance of all sires used by the New York Artificial Breeders Cooperative, Inc., shows the tremendous possibility of herd improvement through breeding. On cows averaging 275 lb. fat, an increase of 79 lb. of fat was obtained. Dams averaging 333 lb. fat had daughters averaging 41 lb. more fat, those averaging 376 lb. had daughters showing a 44-lb. increase, and those averaging 420 lb. had daughters producing slightly less fat than did their dams. The topmost group of dams averaged 505 lb. fat; their daughters averaged 473 lb. Studies by the Bureau of Dairy Industry show that about 0.5 per cent of all sires proved could maintain or increase a 500-lb. fat level. Two bulls in this study were able to do this.

The average butterfat production per cow in New York is about 220 lb. fat. D.H.I.A. herds average around 345 lb. and the average production of the "artificial" heifers is about 415 lb. This startling difference reflects the variation in inherited ability to produce as well as the great differences in environment and opportunity afforded dairy animals.

The Montana Elevated Cow Stall in the Open Shed-milking Stable System. J. O. Tretsven, Montana Extension Service.

In November of 1946, the Montana Agricultural Experiment Station published a preliminary report of research work done on an elevated cow stall used in connection with the open shed-milking stable system of housing dairy cows. This stall was designed to reduce labor to a minimum, to simplify the feeding of grain, to make the milking of cows easier for the operator, to aid in the production of clean milk, and to provide for an easy and rapid exchange of cows through the milking stable. Dairy farmers who are using this type of stall report that it is highly practical. One man, with 2 single-unit milking machines in a 4-stall setup, can milk 20 to 24 cows per hour. This includes bringing the cows into the stall, feeding grain, washing udder and teats, milking, caring for the milk, and turning cows out into the shed again.

The stalls are made of pipe welded together and can be built on the farm at relatively low cost. Four stalls for the large breeds will require a stable 17′ × 17′. With this type of stall, the milker operates between the cows coming in from the head of the stall. The stable is built on two levels to eliminate stooping while milking.

The floor, back of the stalls, and the stall floor is 32 inches above the floor between the stalls, the floor in front of the stalls, and the milkroom floor.

The stalls are only 30 inches wide for the large breeds and are slightly narrower at the head end. To keep the cows quiet when milked, the feed box is set 16 inches above the stall floor and the stanchions are placed 20 inches back from the front of the stall.

The doors for admitting and releasing the cows are operated by the milker from the trenches between the stalls.

All common makes of milking machines may be used.

The Uninsulated Pen Barn in Wisconsin. G. R. Barrett, University of Wisconsin.

A 4-H Dairy Extension Service Program and Plan of Work. H. A. Willman, Cornell University.

Problems which arise in conducting 4–H dairy work may differ widely between states and sections or even within certain states. Therefore, it often is difficult to set up a detailed program carrying with it a set of procedures or standards which may work alike everywhere.

Nevertheless, the entire National 4–H dairy program and plan of work should provide a wide choice of projects and activities. It also should include a proper balance of incentives as a further means of helping to reach, hold, and teach a greater percentage of rural boys and girls some of the fundamentals which underlie efficient dairy production.

The suggestions which follow, while not necessarily complete, are written from the standpoint of the industry and mainly as a general guide in developing state programs. The plan emphasizes the need of youth training as a major problem of the dairy industry.

PROBLEM

I. The development of future leaders, efficient dairymen, and purebred breeders.
  A. Importance of the problem.
    1. Only a very small percentage of dairy herds have been tested regularly, fed adequately, rated for type, analyzed for transmitting ability, or been headed by carefully selected sires. With the future calling for greater dairy farm efficiency, the need of changing from the old to the newer methods and working with youth becomes obvious.
    2. Boys and girls need a well-rounded experience, some income while on the farm, and an early training to prepare them for situations which they may face in later life. Competition from our industrial neighbors constantly will emphasize a need for higher yet efficient dairy production, greater acre yields, and a larger labor output per man per year. The apparent low level of human nutrition also calls for a program directed toward an improved diet of animal food products and expanded markets for these products.

II. Solution to the problem.
  A. A more active interest on the part of parents, the full cooperation of breeders, and the development of programs will help:
    1. Acquaint more boys and girls with the economic importance of dairy cattle as a source of farm income.
    2. Teach the fundamental importance of good dairy type and inheritance, the use of enough feed, proper management, adequate high yielding feed crops, and cattle health.

3. Demonstrate the use of practices which are followed by the more efficient dairymen and breeders.
4. Encourage an income ownership in cattle through 4-H club projects.
5. Encourage a wider use of animal food products in the diet for better health and expanded markets.

III. Plan of activities.

A. Sell the idea of starting more farm boys in dairy club work by using the radio, news articles, folders, posters, and by talks at schools and farm meetings.

B. Enroll boys and girls in the established 4-H projects encouraging the use of cattle which have been selected soundly on a basis of high production inheritance and acceptable type.

C. Visit or contact members at least once annually and keep in touch with county and state breed clubs and leading herd owners.

D. Organize county adult leader-breeder committees as a means of getting help in developing programs, locating suitable calves, testing herds, breeding cattle artificially, visiting project members, enlisting breed club help, and securing qualified people to teach subject matter at meetings.

E. Organize local clubs at which some special instruction may be given by such persons as successful dairymen, veterinarians, older 4-H members, extension dairymen, cow testers, technicians, and others.

F. Hold local, county, district, and state meetings, contests, and shows to stimulate interest, to recognize the achievements of members, and to encourage greater effort.

G. Assist in the development of some of the following national activities as an aid in the building of stronger county and state programs:

1. Judging.
2. Records and management.
3. Demonstrations.
4. Exhibiting, fitting and showmanship.
5. All-round achievement.
6. Cooperative effort.

THE FORTY-SECOND ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. Stoltz, *Secretary-Treasurer*

The American Dairy Science Association assembled in the War Memorial Hall at the Ontario Agricultural College, Guelph, Ontario, Canada, on Tuesday, June 24, at 9:30 a.m.

G. E. Raithby, local chairman, introduced President W. R. Reek, of the Ontario Agricultural College, after an organ prelude and the singing of "The King" and "America". President Reek gave the welcoming address.

Association President Fordyce Ely then was presented and gave the following address:

THE PRESIDENT'S MESSAGE

First, may I take a moment to acknowledge the remarks of President Reek, of the Ontario Agricultural College, who has so graciously welcomed us to the campus. We have been here only a short time but we feel the sincerity of the welcome which has been extended to us by members of the staff of this institution. His welcome starts this Forty-second Annual Meeting of the American Dairy Science Association in an atmosphere of good fellowship which, I am sure, will prevail throughout our sessions. There are many of us who are visiting this beautiful campus for the first time and I know that we will return to our respective institutions with a warm spot in our hearts for the Ontario Agricultural College, our gracious host on this occasion. For several months I have been very grateful for the invitation which was extended our Association to meet here, and I know that I voice the thoughts of all the members present at this meeting when I express our thanks for the many courtesies that have already been extended us as we have assembled.

It seems quite fitting and proper in these times of international intrigue and suspicion that the American Dairy Science Association should meet in Canada. I started to say foreign soil; the situation, however, offers us an opportunity to reflect for a moment on how foreign is this soil of Canada; we certainly do not feel like strangers in a strange land. We have all heard it said that the boundary between Canada and the United States is the longest in the world on which a soldier has never stood guard and across which a malicious gun has never been fired. For reasons best known to others, and which we do not need to discuss here, there has always been a freer trade in education and ideas than in goods and services between Canada and the United States. This reciprocity of thoughts and ideas, in my opinion, is a much more valuable type of free trade than any other that can exist between people and nations. Everything worth while begins with an idea in someone's mind and when these are freely exchanged between people and nations there comes mutual respect and mutual understanding and a high degree of tolerance of each other's rights. It makes one wonder if the lack of this is one of the main things wrong with our world today, as nations suspect each other and for that reason find it impossible to be tolerant of each other's views. This meeting of the American Dairy Science Association is only another manifestation of this free exchange of thought.

During the next 3 days there will be much discussion on what is new in dairy science. The papers and reports given here, together with the private exchange of ideas between those who are interested in similar fields of work, will greatly influence the work in these fields of endeavor, not only for the coming year but for years to come.

Looking Back

Many of us will recall quite vividly a paper given by Dr. Weaver, of Michigan, before a general session of our summer meeting in 1944. I recall that he traced a parallel graph of cow numbers and milk production and a gradual increase in population in our country since 1900. Now as we think back, since 1900 this country has gone through panic and pestilence, two wars followed by peace, economic upheaval in the form of inflation and deflation, repression and suppression; now someone has said we must be prepared for repercussions. It is very significant, however, that none of these has greatly affected the important place which dairying occupies in our country's agriculture. It was pointed out, you will recall, that there exists almost a total lack of cyclic trends in cow numbers and milk production since 1900. There are more people per cow in our country today than at any previous date. On the other hand, we are told that there are about 16 years between cycles in beef cattle numbers, about 6 years between cycles in hog numbers, and a very short time, indeed, with reference to eggs and poultry. These are branches of agricultural effort that compete with cows for feed. These other branches of livestock are also more affected by economic stress and strain, but at the same time they are more adjustable on short notice than our dairy industry. This stabilizing effect of dairying on the agriculture of our nation and especially on the agriculture of a community is reflected in the people who milk cows for a living. Aside from a few of the very wealthy who pay each other high speculative prices for popularly bred cattle, dairymen are by their very nature conservative. Cows seem to appeal to the conservative minded farmers. Cows are in rhyme with conservation. This unwillingness to plunge exists as a healthy stabilizing force which affects our entire industry.

The War Taught Us a Few Things

We learned several very fundamental things during the war as everyone made the supreme effort to produce much-needed food. We learned, for example, that high prices that were offered for milk stimulated the farmer to greater effort, but did not stimulate the cows very much. We learned that time had to pass before calves and heifers became producing cows and feed crops could be diverted into the mouths of cows. During the war period someone remarked, "Take good care of your cows; it takes longer to grow a cow than it does to build a battleship". We learned that sudden expansion of dairying was almost impossible. We accomplished about a 14 per cent increase in production over a period of 5 years. That was the best we could do in an all-out effort to produce, amounting to about 3 per cent per year under the stress of war need. When you compare this 14 per cent with the tremendous percentage of increase in other branches of agriculture that are more easily adjusted than dairying, then we realize again the stable permanence of our industry.

During the war in our quest for more food we did a little too good a job forgetting about quality, and our standards were noticeably lowered. Right or wrong we lived by the rule that poor milk was better than no milk at the time we needed it so badly. Nineteen-forty-six, following V. J. Day, was a year when everyone sought an opportunity to relax. Rest seemed to be of paramount importance following the effort put forth in producing and processing food to win the war. As we stopped to catch our breath it became more and more apparent that the one who said, "Food will win the war and write the peace", indeed predicted accurately. It was also freely predicted that Europe would need a couple of years to get back on an "eat-as-you-go" basis; we now know the error of this prediction, with more hungry nations than ever before in history. On this side of the Atlantic Ocean we have been singularly blessed with a series of bountiful crop years and we have extended this blessing to Europe in our food shipments. Someone said that for this reason the law of averages is against us for 1947, but we hope and pray that our luck will continue; 1947 looks good so far and the world should be deeply thankful.

We Are Awake in 1947

With 1947 there has come a reawakening; we have caught our breath and we are tackling many problems with renewed vigor. It would be quite out of order for me to attempt in these few minutes to discuss fully the many problems that confront our industry in 1947. I think it would be a fair statement to say that is the real purpose of our meeting. The papers and reports that will be presented are given primarily with that thought in view; I will merely make a brief mention of a few.

Renewed Efforts on Quality

Nineteen-forty-seven has brought us a renewed interest in quality standards for milk and other dairy products. With the help of members of our Association, regulatory agencies have perfected their techniques for measuring quality and they are prepared to act in the interest of the public in more adequately enforcing quality standards. Nothing could be more fundamentally sound at this time than aggressive action on the part of our industry in the field of quality appreciation, both with suitable price differentials to farmers and possibly more public education through the use of consumer grades on dairy products other than milk. All branches of the food industry face this same problem, but in our dairy industry it requires a lot of effort to bring order out of a condition which may be described as being more or less chaotic. I am of the opinion that the commercial dairy interests are more serious than ever before in their desire to solve quality problems. I also doubt if there is a single member of the staff of a dairy department here who has not been called into consultation with the commercial interests where help has been sought to devise workable plans for solving local quality problems.

Farmers Help—In Sales

Farmers are learning to realize their responsibilities not only with the quality problem but in many other problems dealing with sales and surpluses. We cannot say how much will be accomplished; probably only a step in the right direction can be accomplished at a time, but the work of the American Dairy Association and their slogan of "sales not surpluses" is, of course, primarily a farmer movement with the support of the commercial dairy interests in collecting over a million dollars to support the program. This is a step in the right direction and exists as another illustration of cooperation within our industry instead of the old give and take which has too often existed between farmers and the men who buy their product.

A Shortage of Trained Personnel

Nineteen-forty-seven has brought to our industry, and especially to our college dairy staffs, personnel problems that are causing gray hairs to sprout. There have been many staff changes during 1946–47; to move seems to be the order of the day. This, with difficult housing facilities in college communities, makes the problem indeed a serious one. There has never been such a shortage of trained personnel in the field of dairying as there is today. This fact challenges members of our Association who are charged with the responsibility of training new workers and teachers. Commercial branches of our industry are also short of this same type of trained worker, and as a result they lean very heavily on members of our dairy staffs who are frequently in a position to be helpful to them in meeting emergency situations. We welcome this close tie-up with our industry if it does not require too much of the time which should be devoted to our own prescribed duties.

College and Breed Associations Are Closer Together Than Ever Before

There exists a finer, more complete working relationship between our colleges and extension staffs and the breed organizations than ever before.

This is not something that just happens, however; it is the product of a gradual, constant, and everlasting effort on the part of members of this Association through the Breed Relation Committee and the Dairy Cattle Breeding Committee, and leaders of the respective breed organizations. I am very favorably impressed with the program of the Purebred Dairy Cattle Association as planned, and with the efforts which are being made by that group to work with committees of this Association. Already much has been accomplished in standardizing testing procedures and the rules under which the artificial breeding program may be conducted.

There are found very few minor difficulties between the breeds in their classification program. The minor differences only exist in the efforts of one or more breeds to be a little more progressive as they initiate new programs. The development of the artificial breeding program is largly a product of the membership of our Dairy Science Association, coupled with the encouraging sanction of these same breed organizations through their Purebred Dairy Cattle Association. More than a million cows are now signed up by organized artificial breeding associations, and I think our dairy industry should be everlastingly grateful to E. J. Perry, of New Jersey, who was mainly responsible for initiating the program that is operating in this country at present. There has been much productive research in this field, but we still know comparatively little about the proper nutrition of bulls, and the last word has not been said or written with reference to diluters. Just a little more productive research in these fields would further advance our methods in conducting the artificial breeding work that has already experienced revolutionary changes within the short period of 9 years.

Some Movement South

With 1947 there has come a new sense of responsibility toward dairying as a source of agricultural income in the South. With the decentralization of industry which is taking place and the movement of some of this industry into the South there has come a new and greater demand for dairy products and the same for cows. There are a few advantages for dairying in the South and also in the same breath we might mention some disadvantages; I will not dwell on them. The fact remains, however, that there is a definite shift of our industry into Southern territory. Without exception leaders in ten Southern states have reported that the increase in dairying is not a temporary thing, but a permanent movement. In eight of ten Southern states development of dairying has been at the expense of "row crops" in general and cotton in particular. Dairy-mindedness seems to be the order of the day among the young people who have not had an opportunity to become so cotton-minded as some of the old timers. Money has been appropriated by Legislatures of a number of the Southern states with the definite objective of developing dairy-mindedness among the youth of their respective states. A visit to some of the 4-H Club dairy shows that have developed in the Southern states is very convincing and serves to illustrate the enthusiasm with which many Southern farmers are accepting dairying as a substantial backlog in their farming plans. Artificial breeding has just begun to reach into the Southern states; suffice it to say that these states are very serious in their intentions along lines of conservative dairy development, and I urge some of our Northern friends not to sell the South short as they consider future dairy developments in this country. Worthwhile developments in any field of endeavor do not occur over-night; the change will come gradually as fundamental difficulties are overcome.

As I end these remarks I am mindful of the many standing and special committees which have faithfully functioned during the past year. As one becomes more familiar with the workings of our Association, one is impressed with the efficiency of our faithful Secretary and the effort and thought which he puts on the affairs of our Association. The American Dairy Science Association is now a big business enterprise with total assets just short of $50,000, with a net profit for the year of nearly $5,100. Things come from or go through the Secretary-Treasurer's office with machine-like precision, and I think that we all should appreciate the value of the services which are rendered by this office. During the year Dr. F. E. Nelson, of Iowa State College, assumed the editorship of our JOURNAL. While we were struggling to find a new editor, several of us wondered if we were actually ever going to be able to find a man who would give as devoted and complete a service as our former editor, Dr. T. S. Sutton. Dr. Nelson has taken over the duties very quickly and my private opinion is that if anyone can make us forget Dr. Sutton's services, it is probably our present editor.

You will be interested and gratified, I am sure, in reports of Dr. Nelson and Professor Stoltz, your Secretary-Treasurer. Many will be very much interested in an announcement which I believe our Secretary will make of a new award to workers in the field of nutrition, closely resembling the Borden Awards. How do you like the News Letter, a product of our new editor's fertile brain?

My tenure of office during the past year has been an enjoyable experience, and I am deeply grateful to the other officers and dirctors, as well as the members of standing and special committees, who actually did the bulk of the work.

The Honorable George A. Drew, K.C., Premier of the Province of Ontario, was then introduced and gave a most instructive address.

Mr. Roberts Everett announced the purpose of the Dairy Industries Society International.

There were 435 members present.

GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Guelph, Ontario, Canada, June 26, 1947

President Ely called the meeting to order at 3 p.m. in the War Memorial Building. There were 166 present.

EDITOR'S REPORT

During 1946 the twelve scheduled issues of the JOURNAL OF DAIRY SCIENCE, constituting volume XXIX, were published under the editorship of Dr. T. S. Sutton. This volume consists of 928 pages of original articles, announcements, proceedings of the annual business meeting, and indices, and 226 pages of abstracts and abstract indices. The total number of JOURNAL pages devoted to material other than advertising was 1,154.

The material published included 82 original papers (42 in the field of dairy manufacturing and 40 in the field of dairy production), 102 abstracts of papers presented at the Annual Meeting, and 447 abstracts of literature in the Abstract Section.

Between January 1, 1946, and December 31, 1946, 87 papers were received. Of these, six were rejected. The number of unpublished papers on hand at the end of the year was 25.

At the time the editorship was assumed by the present incumbent, E. G. Hastings and P. F. Sharp asked to be relieved of their duties as Associate Editors. W. E. Petersen has found it necessary to resign as Associate Editor of the Abstract Section. The editor wishes to express the appreciation of the American Dairy Science Association for the fine work of these men on behalf of the JOURNAL. E. P. Reineke and G. H. Wise have consented to act as Associate Editors of the JOURNAL and already are at work.

The editor wishes to acknowledge the fine cooperation of those people who have contributed, in many instances anonymously, to the publication of the JOURNAL OF DAIRY SCIENCE.

In April the first issue of the News Letter was prepared and mailed to all of our members and student affiliates. A second issue was mailed in June. The favorable response to this new publication moves the editor to ask for approval for continued publication.

Upon motion duly seconded the report of the Editor was approved unanimously.

SECRETARY-TREASURER'S REPORT

Membership and Circulation. The report of our membership and total circulation for the past fiscal year and the first half of 1947 excels any previous 18-month period. At the close of 1946 our total circulation was 2,877, which is over 400 higher than the highest circulation which our JOURNAL has had since its beginning. The former peak was reached in 1941. As of June 1, 1947, our total circulation is 3,508, which is more than 1,000 higher than the previous peak of 1941. Our membership as of June 1, 1947, has reached 1,602, our subscribers 1,292, and we now have 546 student affiliates, which is 150 more than the peak in 1941. The following table gives you the total circulation as of December 31 for the past 10 years and as of June 1 this year.

Dec. 31	Members	New members	Subscribers		Associate sub-scribers	Student affiliates	Total
			Domestic	Foreign			
1937	928		360	385	110	30	1813
1938	1101	332	352	395	125	81	2054
1939	1465	419	321	385	73	156	2400
1940	1339	105	331	360	62	283	2375
1941	1321	103	353	316	66	391	2447
1942	1276	100	371	312	49	235	2143
1943	1324	134	463	208	85	108	2188
1944	1388	111	519	233	81	45	2266
1945	1407	105	586	291	83	32	2399
1946	1583	223	677	350	85	182	2877
June 1, '47	1602	90	805	487	68	546	3508

The following data are a summary of our loss and gain in members for the year 1946:

Membership, December 31, 1945		1407
Gain: New members, 1946	228	
Former student affiliates	14	
Total gain	242	
Loss: Members resigned and delinquent for 1946:		
Delinquent	35	
Resigned	27	
Deceased	4	
Total loss	66	
Net gain in membership		176
Membership, December 31, 1946		1583

You will be interested to know that this year over 75 per cent of our members paid their dues previous to January 1.

Our circulation by states as of June 1, 1947, is as shown in the table on page 607. In computing the total circulation in this table, the new members as listed in the second column are included in the first column also and consequently are not included in the total column.

The states with the highest membership are:

First—Ohio	165	Third—New York	147	Fifth—Wisconsin	95
Second—Illinois	157	Fourth—Pennsylvania	109	Sixth—California	71

State	Members	New members	Sub-scribers	Associate sub-scribers	Student affiliates	Total
Alabama	6	1	9			15
Arizona	11	5	3			14
Arkansas	2		6	1		9
California	71	3	70	6	3	150
Colorado	10		14		1	25
Connecticut	20	3	7		16	43
Delaware	2		5			7
Dist. of Columbia	35	1	10	1		46
Florida	18	4	18		10	46
Georgia	21	1	13	1	34	69
Idaho	8		3			11
Illinois	157	8	64	2	7	230
Indiana	40		21	1	11	73
Iowa	37	4	6		71	114
Kansas	14	3	9		2	25
Kentucky	8		11	2	15	36
Louisiana	14	1	12		5	31
Maine	2		3	1		6
Maryland	38		11	1	3	53
Massachusetts	37		27	6	26	96
Michigan	56	1	20	4	11	91
Minnesota	59	9	15	3	8	85
Mississippi	11	1	4		14	29
Missouri	43		16	1	21	81
Montana	12		4		3	19
Nebraska	13	1	10		11	34
Nevada	1		1			2
New Hampshire	11	1	3			14
New Jersey	36	3	27	2		65
New Mexico	3		4			7
New York	147	5	108	8	13	276
North Carolina	17	3	8			25
North Dakota	4	2	3			7
Oklahoma	18	5	9		24	51
Ohio	165		48	9	58	280
Oregon	20		9		19	48
Pennsylvania	109		44	6	31	190
Rhode Island	3	1	4		2	9
South Carolina	9		5		11	25
South Dakota	7	1	4		7	18
Tennessee	17	3	12	3		32
Texas	30		35		27	92
Utah	9		7		5	21
Vermont	15		5		1	21
Virginia	30		17	2	8	57
Washington	37	2	19	2	12	70
West Virginia	16	1	2	2		20
Wisconsin	95	7	37	1	56	189
Wyoming	4		3			7
Canada & Foreign	54	10	487	3		544
Total	1602	90	1292	68	546	3508

The states with the largest number of subscribers are:

First—New York	108	Third—Illinois	64	Fifth—Pennsylvania	44
Second—California	70	Fourth—Ohio	48	Sixth—Wisconsin	37

The states with the largest number of student affiliates are:

First—Iowa	71	Third—Wisconsin	56	Fifth—Pennsylvania	31
Second—Ohio	58	Fourth—Georgia	34	Sixth—Texas	27

Student Branches. Certificates have been issued to Student Branches of the American Dairy Science Association at the following schools during the past year:

University of Connecticut
University of Georgia
Iowa State College
University of Kentucky
University of Massachusetts
University of Missouri
Washington State College
Cornell University
Oregon State College
Pennsylvania State College
University of Tennessee
Texas Tech. College
Virginia Polytechnic Institute
University of Florida

Back Copies. During the past year the income from the sale of back copies amounted to $2,032.46. We have not reproduced any numbers this past year. We do not have complete volumes of nos. VI, XI, or XXIX. About eleven numbers of our back copies should be reproduced when printing costs are more favorable.

Advertising. The income from advertising during the past year is the greatest income which we have ever had. The number of pages of advertising during the past year was 188, which is slightly less than last year. The income from advertising for 1946 was $7,479.43.

We are pleased to take this opportunity to again express our grateful thanks to the companies that use our JOURNAL as an advertising media. Any courtesies shown our advertisers will be greatly appreciated.

Financial. The income for 1946, amounting to $25,970.04, is the largest income during any year of our organization. Our expenses, which also excelled previous years, were $21,487.60, leaving us an operating profit of $4,483.34 as of December 31, 1946. Our net worth was $35,105.51, which is $5,097.34 higher than last year's net worth. We had an investment in government bonds of $30,799. A complete report of the Certified Public Accountant was sent to each Officer and Director on March 19, 1947.

The Secretary wishes to take this opportunity to express his gratitude and thanks to the officers and members of the organization for the splendid cooperation that they have given him.

Respectfully submitted,
R. B. STOLTZ, *Secretary-Treasurer*

Upon motion duly seconded, the report was approved.

AUDITING COMMITTEE REPORT

The President then requested the report of the Auditing Committee, which was given by Mr. W. E. Krauss.

April 7, 1947

To the Directors and Members of the
American Dairy Science Association

Gentlemen:

The Auditing Committee of the American Dairy Science Association met with Mr. Walter C. Burnham, the certified public accountant who examined the financial statement of the Secretary-Treasurer of the American Dairy Science Association. The Committee met with Mr. Burnham in Columbus on April 7, 1947.

Mr. Burnham stated that he had made a careful audit of the accounts, checked the bank statement and reconciled the checking account. He also stated that he counted the bonds held by the Association.

After conferring with Mr. Burnham, the Committee is satisfied that the statement submitted by the Secretary-Treasurer is correct. We recommend that it be accepted by the Board of Directors and the members of the American Dairy Science Association.

Respectfully submitted,

O. F. Garrett, R. A. Larson, W. E. Krauss

Upon motion duly seconded, the report of the Auditing Committee was accepted and ordered filed.

REPORT OF THE EXTENSION SECTION

The first session of the Extension Section was called to order at 1:00 p.m., June 24, by Chairman W. T. Crandall. Due to the resignation of Arthur Porter as Secretary, the Chair called for nominations to fill this office, and G. Heebink was elected. The chairman appointed Loveland, Harris, and Heebink to the Resolutions Committee and L. A. Johnson, Vergeront, and Burke to the Nominating Committee.

The report of the Dairy Records Committee was given by J. F. Kendrick and Arthur Porter. An interesting report on Canadian Registration and Production Policies was given by G. E. Raithby. R. R. Starbuck discussed Dairy Service Co-operatives in Ohio. A plan for estimating the yield of pasturage on farms was presented by R. E. Hodgson and Floyd Arnold. Fifty-nine people from 27 provinces and states registered at this session.

At the second session a film entitled "A Challenge to New York Dairymen," was shown, and exhibits presented by Ontario, New York, Iowa, Pennsylvania, Missouri, Michigan, Montana, New Hampshire, and California were examined and discussed.

The third session was held jointly with the Production Section. To avoid duplication in reporting this session, the Production Section will cover the report of the Dairy Cattle Health Committee, which was adopted.

The report of the Dairy Cattle Breeding Committee, which consisted largely of recommendations to the Purebred Dairy Cattle Association and pertained chiefly to methods of proving sires and of approving artificial breeding organizations and inseminators, was adopted.

The Breeds Relations Committee's report, which covered recommendations in regard to uniform rules for official testing, D.H.I.A. testing, and for the proving of sires, was adopted.

The symposia on health and the national testing program, in which the Purebred Dairy Cattle Association cooperated, generated much interest and discussion.

At the session on Thursday morning Ray Albrectsen presented a paper on the Selection of Sires for Artificial Breeding Associations. Resolutions pertaining to universal pasteurization of milk and to interstate traffic in cattle infected with Bangs disease were offered and rejected. A resolution regarding thyroprotein was adopted. J. A. Nelson read a paper, prepared by J. O. Tretsven, on the Montana Elevated Cow Stall. The Uninsulated Pen Barn in Wisconsin was discussed by G. R. Barrett. Gearhart and Stallard exhibited and discussed new types of D.H.I.A. equipment.

The final session was devoted largely to a discussion of 4-H Club work, which was led by G. W. Vergeront and J. C. Nageotte.

The report of the Nominating Committee, which nominated C. W. Reaves of Florida as Secretary of the Extension Section, was adopted. The officers for the coming year are as follows: E. H. Loveland, Chairman, G. Heebink, Vice-Chairman, and C. W. Reaves, Secretary.

Respectfully submitted,
FLOYD ARNOLD, *Chairman*
E. H. LOVELAND, *Vice-Chairman*
G. HEEBINK, *Secretary*

Upon motion duly seconded, the report was accepted.

REPORT OF THE MANUFACTURING SECTION

The programs for the Manufacturing Section were held during the afternoons of Tuesday, June 24, Wednesday, June 25, and Thursday, June 26, with C. L. Hankinson as General Chairman.

Thirty-five papers were accepted for the program and only one (M6) was not presented. In addition to the regular papers, three invitational papers were given.

The business meetings of the Manufacturing Section were held Wednesday afternoon and Thursday morning.

The Committee on Reorganization and Records, with S. T. Coulter as Chairman, reported a proposed constitution and by-laws for the Manufacturing Section, which was accepted.

Article I

Name

This organization shall be known as the Manufacturing Section of the American Dairy Science Association.

Article II

Membership

Any member of the American Dairy Science Association interested in dairy manufacturing shall be eligible for membership.

Article III

Officers

Section 1. The officers of this organization shall be a chairman, a vice-chairman and a secretary.

Section 2. Election of Officers. The officers shall be elected annually at the second business meeting and shall hold office for one year or until their successors are elected. Prior to the first business meeting of the Section, the chairman shall appoint a nominating committee of three members whose duty it shall be at the time of the first meeting to place in nomination one or more members for each office. Any member shall have the privilege of placing other names in nomination for each office at either meeting. It shall be the intent of the organization that the vice-chairman succeed to the chairmanship for the following year; that the secretary succeed to the vice-chairmanship; and that the chairmanship alternate between a man from "industry" and a man from "college".

Section 3. The officers of the organization shall perform the duties generally assigned to such officers, and in addition shall constitute the program committee of the section. In order to provide a permanent file of the records of the section, the secretary at the close of his term in office shall send all section records to the Secretary of the American Dairy Science Association.

Section 4. In the absence of the chairman, the vice-chairman shall assume the duties of the chairman, and in the absence of both, the secretary shall assume the duties of the chairman.

Section 5. Committees. All committees shall be appointed by the chairman. The chairman may be directed by a majority vote of the membership present at any meeting to appoint committees, or may himself make *ad interim* committee appointments for specific duties. The duties of each committee shall be specifically provided for at the time it is appointed. Each committee shall be required to report at the annual meeting on what was accomplished and shall then be discharged of its responsibilities unless retained by a majority vote of the Section, in which case the membership shall be appointed as for new committees.

Article IV

Meetings

There shall be two business meetings each year, which shall be held in conjunction with the annual meeting of the American Dairy Science Association.

Article V

Parliamentary Authority

The rules contained in "Roberts' Rules of Order, Revised" shall govern the organization in all cases to which they are applicable and in which they are not inconsistent to these by-laws.

Article VI

Amendment to the Constitution and By-laws

The Constitution and By-laws may be amended at any regular meeting of the organization by a majority vote.

Standing committee reports were presented and accepted as follows:

(a) Committee on Milk, E. O. Herreid, Chairman (three members of his sub-committee submitted written reports).

(b) Committee on Butter, G. H. Wilster, Chairman.

(c) Committee on Products Judging, G. M. Trout, Chairman.

Motions were passed as follows:

1. That the Committees on Milk, on Butter, and on Products Judging be continued.
2. That proposal be made to the Editorial Board to reconsider the matter of publication of abstracts prior to meetings.

Two new officers elected were E. M. Barker, Vice-Chairman, and D. V. Josephson; Secretary. The officers, therefore, for the coming year are as follows: P. R. Elliker, Chairman, E. M. Barker, Vice-Chairman, and D. V. Josephson, Secretary.

Respectfully submitted,

C. L. Hankinson, *Chairman*
P. R. Elliker, *Vice-Chairman*
J. M. Trimble, *Secretary*

Upon motion duly seconded, the report was accepted.

REPORT OF THE PRODUCTION SECTION

The Production Section held six sessions at which some 60 papers were presented. Because of the large number of papers to be presented, it was necessary to have some of the sessions run concurrently.

In addition, one session was held in conjunction with the Extension Section. Committee reports were made by the Dairy Cattle Health Committee, the Dairy Cattle Breeding Committee, and the Breeds Relations Committee. Two symposia were held, one on "Brucellosis in Cattle" in which Dr. B. T. Simms, Chief of the Bureau of Animal Industry, discussed the various problems of the control and eradication of the disease. The subject of the second symposium was "Getting the Most Out of Our Nation-wide Testing Program". The paper prepared by G. A. Bowling entitled "The Testing Program from the Standpoint of the Purebred Dairy Cattle Association" was read. C. T. Conklin discussed "Increasing the Usefulness of the Testing Program to the Dairy Cattle Breeders". Floyd Johnston discussed "The Responsibilities in the Testing Program". C. W. Turner and R. W. Graham discussed "Thyroprotein and Official Testing".

The following reports were presented and accepted by the Production and Extension Sections in joint session.

The Report of the Dairy Cattle Health Committee covered the various aspects of dairy cattle health. The subjects of Brucellosis, Tuberculosis, Foot and Mouth Disease, Johne's Disease, Interstate Cattle Shipments, Breeding Troubles, Calf Raising, Mastitis, Greater Milk Sales, Longer Production Life, and Herd Management were considered. The report concludes "That the Colleges and Experiment Stations remain the bulwark of research and information. The Extension Service and the Veterinary profession provide the field medium of health education distribution. Keeping abreast of the problems, finding their solutions and adequately disseminating the facts is the herd health challenge we face today and in the future." Resolutions on Foot and Mouth Disease and Johne's Disease were drawn for the acceptance by the general session of the Association.

The Report of the Dairy Cattle Breeding Committee was read and accepted.

A Report of the Activities of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association was read by Dr. H. A. Herman. The purpose of this council is to give greater encouragement, in cooperation with experiment stations and colleges, to research in dairy cattle breeding in its broadest phases, such as production, reproduction, type, and economics of dairy farming.

The Report of the Breeds Relations Committee was read and accepted.

In addition to the preceding committee reports of the combined sections, the following reports were read and accepted by the Production Section in a separate business session.

The Committee on Resolutions of the Production Section consisted of I. W. Rupel, L. O. Gilmore, and J. H. Hilton, Chairman. Their report was read and accepted. Two resolutions were adopted for the consideration of the general session.

It was voted to consider the possibilities of publishing the paper given by Dr. B. T. Simms, on Brucellosis, in the JOURNAL OF DAIRY SCIENCE. This paper was given at the Joint Symposium of the Production and Extension Sections.

The Dairy Cattle Judging Committee Report was made by R. E. Johnson. Consideration was given to the awarding of the judging scholarships. It was voted to leave the matter to the discretion of the Awards Committee.

The Nomination Committee of the Production Section consisted of H. P. Davis, G. W. Salisbury, and J. P. LaMaster, Chairman. Nominations for

Secretary were presented. G. M. Cairns, of Maryland, was elected, making the officers for the coming year as follows: G. H. Wise, Chairman, L. A. Moore, Vice-Chairman, and G. M. Cairns, Secretary.

Respectfully submitted,
D. M. SEATH, *Chairman*
G. H. WISE, *Vice-Chairman*
L. A. MOORE, *Secretary*

Upon motion duly seconded, the report was accepted.

The minutes of the Executive Board were then read, and upon motion duly seconded, they were adopted unanimously.

NATIONAL RESEARCH COUNCIL REPORT

The most important action taken by the Division of Biology and Agriculture, National Research Council, with which the American Dairy Science Association is affiliated, concerned the establishment of an Institute of Biological Sciences. In my report a year ago I called attention to the proposed establishment of an Institute of American Biologists. During the past year progress has been made in the development of this Institute, which, by the way, has been renamed the "Institute of Biological Sciences", since the old name implied a narrower coverage of fields than the new one. The National Research Council assigned funds to assist in starting this organization and on March 18, 1947, a selected advisory committee with E. G. Butler, of Princeton University, as chairman, met in the National Research Council Building, Washington, D. C., to discuss procedure for organizing the Institute. At this meeting Dr. H. B. Steinback, of Washington University, consented to act as Temporary Executive Secretary. This committee took the following action: "This committee recommends the establishment of an organizing board for the American Institute of Biological Sciences to be made up of one representative from each of the member societies of the Division of Biology and Agriculture and to include the members of the Advisory Committee".

Dr. Griggs, chairman of the Division of Biology and Agriculture, was instructed to call a meeting of an organizing board as suggested by the Advisory Committee. It was held in the National Research Council Building, Washington, D. C., at which time a proposed constitution and budget for the first year were accepted. These, together with a statement of specific services this Institute might render its members, are attached.

Besides the usual reports of its committees, which were made to the Division of Biology and Agriculture during the year, no other action need be noted.

Respectfully submitted,
C. Y. CANNON

Upon motion duly seconded, the report was approved.

INTER-ASSOCIATION COUNCIL ON ANIMAL DISEASE AND PRODUCTION REPORT

During the past year the Council held a meeting in November at Chicago. The program, "Protecting and Promoting the Livestock Industry", was reviewed. Because of the delays encountered in obtaining approval from the member associations, the Council had been unable to activate the program. In the meantime, however, the interest created by the proposals as they were considered by the membership of the associations had served to provide wide acquaintance with the program. As a result, practically all of the proposals were being studied by appropriate committees of the Agricultural Board of the National Research Council. Consequently, the Council felt it would not be advisable to attempt to duplicate such committees and tabled the program as having accomplished the desired action.

The need for a more comprehensive study and program on Brucellosis in all its aspects is being met by the appointment of a new committee of the National Research Council.

One of the most important subjects discussed at the annual meeting was the shortage of Animal Scientists. Actually, this represents only a portion of the Nation's shortage and the Association's attention is directed to the National Research Council Reprint and Circular Series no. 127, April, 1947, on "The Shortage of Professional Workers in Agriculture and in Forestry". The estimated need for new men with Ph.D. training will total 5,080 in the decade 1946–1955. This is approximately 50 per cent larger than the number now engaged in these fields. To meet the demand, concerted action on a broad front is indicated and the Council favored the formation of an Institute of Biologists. Rarely is one association large enough to meet its own needs for public relations programs but the pooling of resources of all associations would provide for satisfactory action without undue strain upon small individual associations.

The Council recommends that the member associations continue to have symposia at their annual meetings in order that subjects of common interest to two or more associations will be discussed from the widest possible points of view.

Due to the pressure of responsibilities as head of the Agricultural Research Administration, U.S.D.A., Dr. Lambert found it necessary to resign. The American Society of Animal Production has appointed Dr. E. A. Livesay, West Virginia University, to fill Dr. Lambert's unexpired appointment.

The Council recommends that the member associations continue their financial support on the same basis as established earlier.

Respectfully submitted,
W. E. PETERSEN

Upon motion duly seconded, the report was approved.

NECROLOGY COMMITTEE

Ernest H. Brancroft, of Barre Town, Vermont, died December 17, 1945, soon after returning home from the 29th annual convention of the National Cooperative Milk Producers' Federation, of which he was a director. Dr. Brancroft was graduated from the Chicago Veterinary College in 1911. He was instrumental in the founding of the Granite City Cooperative Creamery in 1920 and was active in the dairy affairs of Vermont the remainder of his life. He was interested in politics. In 1925–26 he was a member of the Vermont House of Representatives and from 1929 to 1932 he was in the Vermont Senate. Dr. Brancroft is survived by Mrs. Brancroft, seven children, and four grandchildren.

F. W. Burns was born at Wayne, Illinois, in 1896. He went to Alabama Polytechnic Institute in 1917, where he taught for 7 years, becoming dairy specialist of the Alabama Agricultural Extension Service, which position he held until his death on April 11, 1945, following a heart attack while on a pasture tour. Professor Burns was active in state, community, and church affairs. He is survived by Mrs. Burns, a daughter, and a sister.

Forrest Clifton Button, 52, of Highland Park, New Jersey, died unexpectedly at his home, May 27, 1947, after a heart attack. He was working in his garden when he became ill. He was graduated from the College of Agriculture at Cornell University in 1916, and he received the Master's degree from Cornell University in 1932. Mr. Button was professor of dairy manufactures at the New Jersey College of Agriculture. He was interested in many civic, social, scientific, and religious organizations. During World War I he was with the U. S. Health Department. He is survived by his wife, a son, and two brothers.

Oscar Erf, 72, died at the home of his son, Dr. Lowell A. Erf, of Philadelphia, Pennsylvania, on April 28, 1947. He was a native of Monroeville, Ohio. He received his first college degree from Ohio State University in 1899. He later studied at the Von Behring Physiological Institute in Germany, and was awarded an advanced degree in 1903. Before being called to head the Department of Dairying at Ohio State University in 1907, he served in that position for a few years in the Kansas State College of Agriculture and Applied Science. He became professor emeritus in 1940 and was retired in 1945. Professor Erf was one of the founders of Alpha Zeta, a professional agricultural fraternity, and had served as Vice-President of the American Dairy Science Association.

Joseph N. Hopkins, Chief of the Dairy Products Laboratory of the State Department of Agriculture, died unexpectedly in Raleigh, North Carolina, on March 29, 1947. Immediately on release from the U. S. Army he had accepted this position. He was graduated from the Virginia Polytechnic Institute in 1932, and he was granted the degree, Master of Science, by Ohio State University in 1936. He is survived by Mrs. Hopkins, three daughters, his parents, and one sister.

Samuel D. Isaly, 47, died suddenly of a coronary occlusion in Phoenix, Arizona, on December 3, 1946. He was born in Mansfield, Ohio, and was graduated from the University of Chicago in 1920. For the past several years he was president and manager of the Isaly Dairy Company of Youngstown, Ohio, and the Secretary-Treasurer of the Isaly Dairy Company of Pittsburgh, Pennsylvania, a separate company. He also was an officer and a director of the Isaly Dairy Companies in Mansfield, Marion, and Columbus, Ohio. Mr. Isaly was active in local dairy and civic affairs as well as being interested in several national and international dairy organizations, among them being the American Dairy Science Association. He is survived by Mrs. Isaly, a daughter and son, a brother, and two sisters.

Charles D. Miller, of the Miller Ice Cream Company, Eaton Rapids, Michigan, was killed in a private airplane crash December 14, 1946.

Henry Rive, 1881–1946, was born in Jersey, Channel Islands. He was graduated from Toronto University in 1903 with B.S.A. degree, and was instructor in the Department of Dairying, Ontario Agricultural College, 1903–04. Mr. Rive was employed in commercial creamery work in the Province of Ontario from 1905 to 1909. He was appointed Dairy Instructor and Inspector in British Columbia in 1910, continuing until 1919. He saw military service in World War I. Appointed Dairy Commissioner with the Provincial Department of Agriculture, Victoria, B. C., July, 1919, he died just 2 weeks before he was due for retirement at the age of 65 years.

Respectfully submitted,
R. B. Becker
H. S. Willard
E. S. Guthrie

Upon motion duly seconded, the report was accepted.

RESOLUTIONS COMMITTEE

Mr. W. E. Krauss, Chairman of the Resolutions Committee, submitted the following report, and upon motion duly seconded, it was adopted.

Whereas: The Ontario Agricultural College and the Ontario Veterinary College, through their administrative staffs and faculty, have made available to the American Dairy Science Association in this its 42nd Annual Meeting all needed physical facilities for the meeting, and

Whereas: Every possible personal courtesy has been given to members of the Association, and

Whereas: The messages of President Reek and Premier Drew at the opening session of the meeting have been exemplary of the spirit of excellent international relationships and attitudes,

Therefore, be it *Resolved:* That the American Dairy Science Association herewith officially extend its thanks and appreciation to the Ontario Agricultural College and the Ontario Veterinary College, and that the President of this Association express this appreciation by letter to President W. R. Reek, Principal A. L. McNabb, and the Honorable George A. Drew.

WHEREAS: Because of interruption of mail service and lack of funds during and following World War II many institutions and individual workers in foreign countries have failed to receive scientific journals, experiment station and agricultural college publications, and reprints of published articles in the field of dairy science,

Therefore, be it *Resolved:* That the membership of the American Dairy Science Association be encouraged, through institution, department, concern, and individual effort, to supply such literature as the needs of institutions and individuals become known.

WHEREAS: The world food situation is still acute and probably will continue to be for some time, and

WHEREAS: Milk and products derived from it afford a sound basis around which to build satisfactory and economical diets,

Therefore, be it *Resolved:* That the American Dairy Science Association encourage greater and more efficient production of high quality milk.

WHEREAS: The Borden Company has again considered it desirable to offer their awards for outstanding research in dairy manufacturing and production,

Therefore, be it *Resolved:* That the American Dairy Science Association express to the Borden Company its sincere appreciation of this evidence of interest in research which plays such a vital rôle in the progress of the dairy industry.

WHEREAS: The American Feed Manufacturers Association has inaugurated an award to be presented by the American Dairy Science Association for the most outstanding research contribution in the field of dairy cattle nutrition,

Therefore, be it *Resolved:* That the American Dairy Science Association express to the American Feed Manufacturers Association its sincere appreciation of this evidence of interest in research which plays such a vital rôle in the progress of the dairy industry.

WHEREAS: The Purebred Dairy Cattle Association has established a Dairy Cattle Breeding Research Council for the purpose of encouraging and supporting research in the field of dairy cattle breeding in cooperation with federal and state experiment stations, and

WHEREAS: Members of the American Dairy Science Association have been asked to serve on various committees of this council,

Therefore, be it *Resolved:* That the American Dairy Science Association hereby commend the Purebred Dairy Cattle Association for this important forward-looking action.

WHEREAS: Heavy volume production of milk and its products at all times is vital to the nutritional and health requirements of all adults and children of Canada and the United States and to the prosperity of the dairy community generally, and

WHEREAS: Healthy dairy animals are the basis of the vast dairy industries of these countries and are essential to the production and maintenance of this necessary high milk volume, and

WHEREAS: Foot and mouth disease, a dreaded livestock malady, now present in Mexico, if not finally eradicated threatens the welfare of our citizens and our dairy and livestock industries,

Therefore, be it *Resolved:* That the American Dairy Science Association hereby recognizes and points out the seriousness of this catastrophe which has befallen the livestock industries of our neighboring Republic to the South, and

Be it further *Resolved:* That the American Dairy Science Association support the action of the United States Government and its Bureau of Animal Industry actively to cooperate with Mexican livestock sanitary authorities to control and stamp out this disease.

WHEREAS: Johne's disease or paratuberculosis is a chronic, insidious disease of cattle, sheep and goats, which is on the increase in this country and is more wide-spread than is publicly known, and

WHEREAS: This disease is a potential danger to the health of herds, since dependable, practical field tests for diagnosis are unknown in spite of much fundamental research,

Therefore, be it *Resolved:* That the American Dairy Science Association go on record as favoring and encouraging further active research by the U. S. Department of Agriculture and the several states in the field of diagnostic methods which will eventually result in more accurate and more reliable tests that may be extensively applied to prevent and to control Johne's disease, which is now such a baffling problem to dairymen and veterinarians.

WHEREAS: Much of the commercial vaccine used in the program of Brucellosis control is of questionable value when used, and

WHEREAS: There is just cause for much confusion in the minds of farmers, veterinarians, and other leaders who are called upon to advise farmers in their choice of programs for the control of Brucellosis, and

WHEREAS: These conditions jeopardize the results of the program aimed at the control of this difficulty,

Therefore, be it *Resolved:* That the American Dairy Science Association go on record as recommending that the preparation, distribution, and use of vaccine to be used in the control of Brucellosis be placed under the control of the Bureau of Animal Industry of the United States Department of Agriculture.

WHEREAS: The incidence of human Brucellosis is increasing to the point where this disease constitutes a major public health problem, and

WHEREAS: The incidence of human Brucellosis is related to the incidence of bovine Brucellosis,

Therefore, be it *Resolved:* That the American Dairy Science Association recommend that universal pasteurization of the milk and cream supply would be a desirable public health measure and that the dairy industry be encouraged to take steps to bring this practice about, and

Be it further *Resolved:* That the membership of the American Dairy Science Association, through its teaching and extension activities, encourage home pasteurization of the milk and cream supply in rural homes, and in urban homes where pasteurized milk and cream are not now available.

WHEREAS: There is lack of agreement among investigators as to the desirability of widespread use for dairy cattle of thyroid extract, thyroxine, or any product producing thyroidal effects,

Therefore, be it *Resolved:* That the American Dairy Science Association recommend that further research on these products be undertaken and pursued to the point where clarity of results are obtained.

Respectfully submitted,

G. H. WILSTER, G. W. VERGERONT, J. H. HILTON,
K. L. TURK, W. E. KRAUSS, *Chairman*

REGISTRATION COMMITTEE

Mr. John Eccles, Dean of Men, made the following report for the Registration Committee. Upon motion duly seconded it was accepted.

New York	52	New Hampshire	7	Washington	2
Ohio	42	Nebraska	7	Connecticut	2
Illinois	35	West Virginia	7	Kansas	2
Wisconsin	29	Virginia	6	Wyoming	1
Pennsylvania	21	Tennessee	5	Arkansas	1
Minnesota	21	Kentucky	4	Alabama	1
Michigan	19	Quebec	4	Georgia	1
Maryland	15	California	4	Florida	1
New Jersey	14	South Dakota	4	Louisiana	1
Ontario	13	South Carolina	3	Maine	1
Vermont	12	Texas	3	Mississippi	1
Washington, D. C.	11	Rhode Island,	3	Montana	1
Indiana	10	Massachusetts	3	North Dakota	1
Iowa	10	Oregon	2	Cuba	1
Missouri	9	Oklahoma	2	Nova Scotia	1
North Carolina	9	Utah	2	Manitoba	1
					407

Registration of members	407
Registration of non-members	176
Total registration	583
Women and children	328
Total attendance	911

Members from 4 provinces in Canada, 43 states in the United States, and the District of Columbia were in attendance. Non-members attended from Chile, Colombia, Hawaii, India, Holland, Sweden, and China.

Mr. Borland moved and Mr. Davis seconded that all action of the Executive Board during the past year be approved.

MEETING OF THE EXECUTIVE BOARD AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

The Executive Board transacted the following business:

Approved the Editor's Report.

Approved the Minutes of the past Annual Meeting.

Approved the Secretary-Treasurer's Report.

Authorized the Secretary to purchase back copies of the JOURNAL.

Approved the report of the Auditing Committee.

Increased the budget for 1947 from $23,000 to $25,800.

Approved and prepared a budget for 1948 of $28,800.

Received the report of the representative of the National Research Council.

Placed on the table the question of affiliating with the International Dairy Federation.

Approved a plan submitted to handle student affiliates by groups rather than by individuals.

Accepted the proposal of the American Feed Manufacturers to make a $1,000 award for the best paper published on animal nutrition, and authorized the President to appoint a committee to draw up rules for handling this award.

Authorized the Secretary to issue membership receipts only to those members who request them.

Approved the action taken by the Executive Board during the past year:

1. Approval of issuing the News Letter.
2. Increasing the budget of the Secretary's clerical work.
3. Making life members of:

H. A. Harding	H. E. Ross
S. I. Bechdel	C. W. Larson

Approved the Inter-Association Council Report.

Proposals to meet in North Carolina and Georgia were discussed but postponed for later acceptance.

Rejected the recommendation to become a member of the American Institute of Biological Sciences.

Approved the Journal Management Report, which is as follows: Your Journal Management Committee desires to report as follows: Permission has been given to Squibb & Company to reproduce in their Abstract Bulletin the authors' summaries of articles in the JOURNAL OF DAIRY SCIENCE.

At the last meeting of the Association it was recommended that the possibility of making arrangements with the British Abstract Service for use of their abstracts in our JOURNAL be surveyed. The possibility has been surveyed and the plan has been found not feasible. In lieu of the inability to enlarge the abstract service in the above named manner the Committe endorsed the plans of the Editor to rearrange the procedure of handling abstracts and to broaden the coverage of journals.

The Committee endorsed also a study by the Editor of the possibility of arranging the abstracts in two columns on a slightly increased journal page to effect a saving in space.

Requests have been made that the abstracts of the papers presented at Regional Meetings of dairy science groups be published in the JOURNAL OF DAIRY SCIENCE. The Committee agreed unanimously that the request should not be granted. However, the committee recommended that the programs of these meetings be published in the News Letter.

The prompt publication of manuscripts submitted to the Editor is highly desirable. The Committee placed itself on record as favoring issuance of numbers varying in size with that of the amount of material accepted for publication, the size of issues to be left to the discretion of the Editor.

The Committee also recommends that negotiations be entered into with the International Ice Cream Association and the Milk Industry Foundation to release the Editor from the necessity of submitting the abstracts to their representatives for approval for publication.

Approved the Resolutions Committee Report.

Employed Editor for the year 1948. Employed Secretary-Treasurer for the year 1948.

Elected Honorary Member for present meeting.

Elected Paul R. Elliker to the Journal Management Committee.

Recommended the dismissal of the Committee on Quality Milk appointed last year.

The Nominating Committee consisting of A. C. Ragsdale, Chairman, J. A. Nelson, I. W. Rupel, Floyd Johnston, and H. W. Norton, nominated the following officers in April: Vice-President, R. B. Becker, of Florida, and W. E. Petersen, of Minnesota; Directors, James H. Hilton, of North Carolina; Harry A. Herman, of Missouri; Paul F. Sharp, of California; G. H. Wilster, of Oregon.

The results of the election were announced on May 31 as follows: W. E. PETERSEN, *Vice-President;* JAMES H. HILTON AND PAUL F. SHARP, *Directors.*

AMERICAN DAIRY SCIENCE ASSOCIATION ANNUAL BANQUET

Guelph, Ontario, Canada, June 26, 1947

President W. R. Reek, of Ontario Agricultural College, was toastmaster at the annual Association banquet and presented President Fordyce Ely, who installed the officers-elect as follows: Mr. Paul H. Tracy, of Illinois, was installed as President, Mr. W. E. Petersen, of Minnesota, as Vice-President, Mr. Paul F. Sharp, of California, and Mr. James H. Hilton, of North Carolina (both in absentia), as Directors.

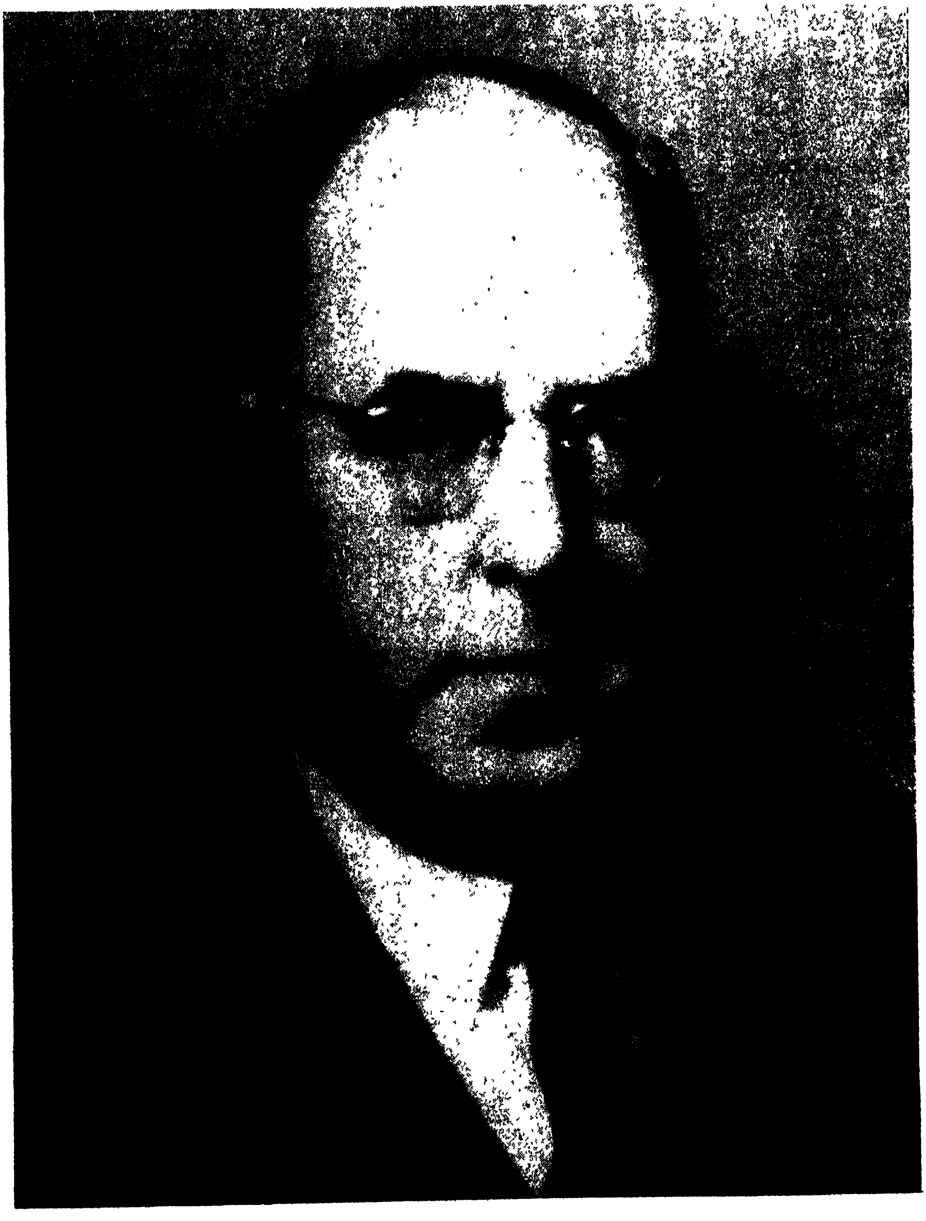
PRESENTATION OF ASSOCIATION AWARD

The toastmaster then introduced Mr. A. C. Dahlberg, Chairman of the Association's Honors Committee. Mr. Dahlberg made the following citation:

Robert Bear Stoltz is a native son of Ohio, educated in the public schools of that state, and graduated from Ohio State University. Immediately after graduation he began his illustrious career in dairy work at Ohio State University. He was made instructor in dairying in 1912, assistant professor in 1914, and professor in 1923. In 1929 he was advanced to the chairmanship of the newly organized Department of Dairy Technology.

Few men in dairy education have had such extraordinary influence upon the dairy industries of their states. He has served as secretary-treasurer of the Ohio Swiss Cheese Association since 1918, during which time the Swiss cheese of Ohio became known for its high qualtiy. He has been secretary of the Columbus Milk Distributors Association since 1933. Through his skillful management, the bottle exchange of this organization became known throughout the nation. He was secretary-treasurer of The National Cheese Association from 1920 to 1925. In 1937 he studied the dairy industries of New Zealand and Australia. Students graduated from the Department of Dairy Technology occupy important positions throughout the dairy industry of this country. The dairy industry of Ohio recognized his leadership and worth to the industry, and recently contributed a special research fund of $100,000 to Ohio State University to be administered by him.

He has attended thirty of the thirty-five annual meetings of the American Dairy Science Association since he graduated from college. He has been honored by having four of these meetings in Columbus. In 1931 he served as Chairman of the Manufacturing Section, in 1932 and 1933 he was Vice-President, and in 1934 he was President of the Association. He assumed the responsibility of Secretary-Treasurer in 1936, and is still making an enviable record in that position. In 1936 the Association had 727 members, a paid JOURNAL circulation of 1,344, and assets of $7,939.86. The report for last year showed 1,534 members, a paid JOURNAL circulation of 2,399, and assets of $30,008.17. This excellent record is a splendid tribute to him.



ROBERT BEAR STOLTZ

It is with great pleasure that we present to Robert Bear Stoltz this certificate of Honorary Life Membership in The American Dairy Science Association.

Signed: A. C. RAGSDALE, J. A. NELSON, A. C. DAHLBERG

PRESENTATION OF BORDEN AWARDS

Mr. W. A. Wentworth, of the Borden Company, was then introduced and in turn presented the Borden Recipients during the past 10 years and their families. He then read the following statement from a brochure, which was given to each guest at the banquet: "The fact that material progress must be soundly based on scientific research is nowhere more evident than in the dairy industry. Milk is at once the most essential and the most complex of foods, backbone of a sound agriculture and a nutritious diet.

"Many of the recent advances in dairy science were pioneered by the men whose works are recorded in these pages. We believe that their accomplishments will serve as an inspiration and a challenge to others, and that the future will mirror as distinctly as the past the contributions to better living made by the members of the American Dairy Science Association."

Borden Award in Dairy Production

Mr. J. B. Fitch, Chairman of the Borden Award Committee for Production, was then introduced and made the following statement:

The Borden Award in Dairy Production for 1947 has been awarded to Joseph Clement Shaw, of the University of Maryland, College Park, Maryland. Dr. Shaw received his B.S. degree at Iowa State College in 1930, his M.S. degree from Montana State College in 1933, and his Ph.D. degree from the University of Minnesota in 1938.

Dr. Shaw has held the following positions since his graduation from Iowa State College: District Club Agent, South Dakota, 1930–32; Research Assistant, University of Minnesota, 1933–38; Associate Physiologist, University of Minnesota, 1938–40; Associate Professor Dairy Industry, University of Connecticut, 1940–45; and Professor of Dairy Husbandry, University of Maryland, since 1945. Dr. Shaw is in charge of the Dairy Production Research at the University of Maryland.

Dr. Shaw's interest and activities have been directed to dairy cattle physiology as it involves physiology of milk secretion, to herd health from the standpoint of ketosis (acetonemia), and also to various aspects of dairy cattle nutrition. His most outstanding contribution to the field of dairy production has been his basic scientific studies on ketosis in dairy cattle and he is recognized as the outstanding authority in this field of work. Through original and fundamental research he established relationships between chemical changes in the blood and the occurrence of this physiological disturbance. He was the first to study the effect of various feeding and man-

JOSEPH CLEMENT SHAW

agement practices on the production of ketosis. In addition to his valuable contributions to our knowledge of ketosis, he has added materially to our fundamental information on milk secretion.

Dr. Shaw has published 26 papers in the field of physiology. Seventeen of this number are in his own name or as senior author. He has published 16 abstracts; eleven are in his own name or as senior author.

On behalf of the Committee on the Borden Award for Dairy Production, it is a pleasure to present Dr. Shaw to receive the Award.

Mr. Wentworth then presented to Dr. Shaw a gold medal and $1,000.

Borden Award in Dairy Manufacturing

Mr. K. G. Weckel, Chairman of the Borden Award Committee for Manufacturing, was then introduced and made the following statement:

The recipient of the 1947 Borden Award in Dairy Manufacturing unanimously chosen by the Borden Award Committee from among a number of very impressive candidates is one having the benefit of broad national geographic experience. He was born in Cheney, Washington, and received his Bachelor's, Master's, and Doctor's degrees from the University of Minnesota in 1920, 1921, and 1937, respectively. His academic training was delayed by military service in the Army Air Force from 1917 to 1919, during which he served with the American Expeditionary Force. He has served as instructor and Assistant Professor at the University of Minnesota, and as Associate, and subsequently Professor, at Pennsylvania State College, where he is head of Dairy Manufactures in the Department of Dairy Husbandry. Dr. Chester Distad Dahle has been successful in, and highly regarded for, his activities in at least three very difficult fields in dairy technology: as an educator, in development research, and in industry service. It is estimated he has supervised the training of approximately a thousand 4-year students in dairy technology, 30 graduate students, and over 2,000 joint industry-college short course students.

In the realm of dairy research, he has studied and published material on a wide range of manufacturing problems. Dr. Dahle has been a pioneer in the study of the behavior of ice cream mixes upon aging; of the manufacture of concentrated ice cream mix; of the high-short pasteurization of ice cream mix; of the "bound-water" of dairy products; of the electro-kinetic velocity of fat globules, and its relation to certain dairy phenomena; of the development of oxidized flavor in fruit ice creams due to metallic effects; and of the nature and effects of removal of fat globule membrane on properties such as the churning, freezing, and thawing characteristics of cream and the whipping ability of ice cream mixes. He has contributed much from his studies on the properties and effects of the several forms of ingredients in ice cream, the homogenization of ice cream mix concerning which he developed a now widely-used homogenizer valve, the production of Cheddar and

CHESTER DISTAD DAHLE

of grating type hard cheese, the production and characteristics of plastic frozen cream, and the properties and uses of dry skim and whole milk. It should not be overlooked that the State of Pennsylvania, from which he hails, is the Nation's largest producer of ice cream.

In addition to his research, Dr. Dahle has been active in the affairs of the Association as a Chairman of the Manufacturing Section, and currently as an Abstract Editor for the JOURNAL OF DAIRY SCIENCE. As Chairman of the Statistical Research Committee of the International Association of Ice Cream Manufacturers, as a regular contributor to the industries trade publications, and as a conductor of successful industry short course conferences, he has ably translated science to the useful benefit of the manufacturing industry.

I have the pleasure to present, on behalf of the American Dairy Science Association, Dr. Chester Dahle, to receive the Borden Award in Dairy Manufacturing.

Mr. Wentworth then presented to Dr. Dahle a gold medal and $1,000.

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THE RELATION OF SEASON OF FRESHENING TO MILK PRODUCTION

GEORGE E. FRICK, A. I. MANN, AND STEWART JOHNSON

University of Connecticut, Storrs

In an effort to determine the relation of season of freshening to milk production, a study was made of 22,212 Dairy Herd Improvement Association lactation records. These records were obtained in Connecticut during the years 1924 through 1945, although the majority of them were obtained in the later years of this period.

EXPERIMENTAL

The amount of milk produced by cows freshening in each month had been computed by the University Dairy Department for the 305 days following the date of freshening. In many instances the cows were dry before this period of time had elapsed and in such cases the production covered a shorter milking period. Data for each breed were tabulated separately. The breed distribution was 9,325 Guernseys, 8,260 Holsteins, 2,344 Ayrshires, and 2,283 Jerseys. The lactation records were sorted not only by breed but also by the age of the cows at time of freshening. Two-year-olds accounted for 22 per cent of the total number of records, 3-year-olds for 21 per cent, and 4-year-olds for 16 per cent. Milk yields were used as reported and were not adjusted to a common butterfat test.[1]

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[1] As milk is priced in Connecticut and in many fluid milk markets of the East, the amount of milk produced is much more important than the amount of butterfat. For this reason the lactation records were not adjusted to give yields of 4 per cent fat-corrected milk. In April, 1947, for example, the price paid Connecticut wholesale producers for 3.7 per cent milk averaged $5.53 per cwt., and the butterfat differential was six cents per point. An increase in butterfat test from 3.7 to 4.0 per cent would bring a price premium of only 18 cents per cwt. A corresponding increase in the volume of milk (8.1 per cent) would bring a price premium of 45 cents per cwt.; an increase in the volume of milk equivalent to the increased energy content of 4.0 per cent milk as contrasted to 3.7 per cent milk (about 5.3 per cent for Holstein cows) would bring a price premium of 29 cents per cwt. Moreover, if the records had been converted to a common butterfat base, the conclusions of the study would not have been modified significantly. For example, yields for Holstein cows in the most favorable month would have exceeded those in the least favorable month by 13.9 per cent, rather than by 14.9 per cent as noted in the analysis of records of actual volumes.

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TABLE 1

Average milk yields, by ages, for 9,325 Guernsey cows freshening in different months

Month of freshening	Age at date of freshening, in years													No. of cases
	1	2	3	4	5	6	7	8	9	10	11	12 and over	All ages*	
	(lb. milk per cow, 305-day lactation period)													
Jan.	5790	6700	7080	7580	8000	8590	8330	8430	8680	8800	8030	8070	7540	825
Feb.	6020	6680	7390	7900	8100	8210	8160	8440	8340	8630	8370	7780	7610	723
March	5550	6660	7210	7520	7870	8270	8640	8240	8530	8530	7960	8520	7500	776
April	5140	6530	6980	7650	7850	8150	8030	8130	7970	7870	9100	8210	7360	712
May	4500	6330	6850	7750	7900	7780	7780	7970	7760	8300	7000	7200	7230	626
June	5160	6110	6640	7220	7070	7220	7330	7380	7210	6970	6850	6200	6800	659
July	5930	6110	6530	6950	6820	7260	7140	7130	6890	6800	5650	6340	6670	783
Aug.	4760	6140	6810	7250	7320	7370	7750	7360	7440	7500	8960		6950	786
Sept.	6120	6130	6750	7230	7520	7500	7310	7710	7410	7410	6300	7250	6950	859
Oct.	6070	6620	7080	7500	7970	7840	8240	7950	8050	8720	7040	8100	7380	928
Nov.	6300	6630	7340	7860	8070	7960	8260	8170	8650	6920	7450	6950	7520	828
Dec.	5560	6610	7110	7940	8190	8510	8690	7740	7520	8760	8460	6840	7550	820
All months†	5580	6440	6980	7530	7720	7890	7970	7890	7870	7930	7600	7410	7260	. .
No. of cases	98	2196	1950	1479	1194	880	612	422	235	133	67	59	9325	9325

* These averages were obtained by weighting the yield for each age group by the total number of cows in that age group regardless of month of freshening.

† These averages were obtained by dividing the 12-month total of the average monthly yields by 12.

RESULTS

Month of calving and milk yields, by breeds. As shown in table 1, of the nine age groups of Guernseys with more than 100 cases, January was the most favorable month in four instances, December in three, and February in two. July was the least favorable in seven of these groups, June in one, and June and July yields were equal in one. The term "favorable" as used here is based on the volume of milk produced by cows freshening in each month. It is not based on the value of these yields, which would be influenced by the seasonal pattern of milk prices, or on the yields relative to costs of production.

For all Guernsey cows, yields were highest for those freshening in February and lowest for those freshening in July. Average production for cows calving in February was 7,610 lb. and for cows calving in July was 6,670 lb. The difference in favor of cows freshening in February was 940 lb. From February until July, average yields decreased for cows freshening in each succeeding month, and from July until February average yields increased from each month to the next.

TABLE 2

Relative importance of various months of calving for Guernsey cows in different age groups

Age at date of freshening, in years	Percentage of total cows freshening during year that calved in:											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1 & 2	9	7	6	7	5	6	7	10	11	11	11	10
3	9	7	8	7	7	6	8	9	10	11	9	8
4	9	8	9	8	6	8	9	7	9	9	10	9
5	9	8	10	9	8	7	8	7	8	11	8	8
6	8	8	10	9	8	7	9	8	7	10	7	9
7	9	9	9	8	8	9	10	7	6	9	8	8
8 & over	10	9	10	9	6	9	8	7	8	8	7	8
All ages	9	8	8	8	7	7	8	8	9	10	9	9

The average yield for all cows freshening in a given month was calculated by weighting the yield for each age group by the total number of cows in that age group regardless of month of freshening. This method was chosen because of the joint relationship of age with season of freshening. A larger-than-usual number of 1- and 2-year-old Guernseys freshened in the fall months but this tendency gradually disappeared as the cows became older (table 2). A simple average, with total production for all cows freshening in a given month being divided by the number of cows, would be influenced by the proportion of cows which were of a young age. If the weighting factor in each age group is the total number of observations for the year, possible errors due to the joint relationship of season of freshening with age are avoided. If weighted averages according to the method described had not been used, February through July yields would have erred on the high

TABLE 3

Average milk yields, by ages, for 8,260 Holstein cows freshening in different months

Month of freshening	Age at date of freshening, in years													No. of cases
	1	2	3	4	5	6	7	8	9	10	11	12 and over	All ages*	
	(lb. milk per cow, 305-day lactation period)													
Jan.	6870	7930	9470	10270	10510	10920	10340	10370	10460	11290	11180	10170	9720	744
Feb.	5600	8480	9830	10520	10900	11040	10990	11780	11330	11140	8320	10920	10120	685
March	7370	8260	9190	10330	10610	11080	10680	11110	9780	11330	9490	9650	9790	691
April	7290	8200	9620	10030	10640	10560	10710	10860	10670	10440	9800	8800	9770	568
May	5880	8010	8520	10120	10100	10740	10930	9710	10490	9680	9830	9120	9390	487
June	7000	8120	8840	9610	9560	9880	9920	10200	9830	9280	9230	11140	9200	549
July	7690	7930	8740	8970	9560	9670	9990	9360	8980	9770	8770	8390	8930	722
Aug.	7330	7740	8120	9460	9450	9360	9650	9210	10000	9880	9240	9320	8810	796
Sept.	8150	8090	9630	9420	9810	10040	10860	9760	9830	10200	9500	9170	9220	777
Oct.	6500	8020	9010	9960	10050	10690	10890	10500	10990	10920	9870	9450	9540	760
Nov.	7400	8440	8900	10060	10580	10340	10690	10380	10750	10650	12400	8750	9650	749
Dec.	8080	8750	9080	9990	10270	10270	10480	9890	11500	10390	11280	11420	9700	732
All months†	7100	8160	9000	9900	10170	10380	10510	10260	10380	10410	9910	9690	9490	
No. of cases	81	1698	1739	1336	1094	787	548	393	249	163	84	88	8260	8260

* These averages were obtained by weighting the yield for each age group by the total number of cows in that age group regardless of month of freshening.

† These averages were obtained by dividing the 12-month total of the average monthly yields by 12.

side, and August through January yields would have erred on the low side; the extent of error would have been as large as 80 lb. in August and September and 110 lb. in March.

Average yields of Holsteins were highest for cows freshening in February and lowest for cows freshening in August (table 3). The most favorable month was the same as for Guernseys, but the least favorable was a month later in the summer. Milk yields were 14.9 per cent greater for cows freshening in the most favorable month than in the least favorable month, slightly greater than the corresponding difference of 14.1 per cent noted with Guernsey cows.

Thirty-three per cent of the 1- and 2-year-olds freshened in the September–November quarter, but this fall-freshening tendency gradually disappeared as age increased, with only 21 per cent of the cows 8 years old and over freshening in this quarter (table 4). This corresponds closely with the association of age with season of freshening as found with Guernsey cows.

TABLE 4

Relative importance of various months of calving for Holstein cows in different age groups

Age at date of freshening, in years	Percentage of total cows freshening during year that calved in:											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1 & 2	9	8	6	5	4	6	9	10	12	11	10	10
3	10	8	8	6	6	5	8	11	11	10	10	8
4	8	8	10	7	6	7	8	10	10	8	10	9
5	10	9	8	7	7	7	9	8	7	9	9	10
6	10	9	9	7	6	7	10	9	9	8	8	9
7	8	9	11	10	5	7	9	9	5	8	8	11
8 & over	7	8	10	10	9	9	10	8	6	8	7	7
All ages	9	8	8	7	6	7	9	10	9	9	9	9

Milk yields were highest for Ayrshire cows freshening in January and lowest for those freshening in July (table 5). Yields in the most favorable month exceeded those in the least favorable by 16.2 per cent.

As with the Ayrshires, milk yields were highest for Jersey cows freshening in January and lowest for those freshening in July (table 5). The difference in yields between the most favorable and least favorable months was greater than for any of the other three breeds; cows freshening in January produced 19.2 per cent more than those freshening in July.

Tests of significance of averages. Although there was considerable dispersion about the average milk yield for cows freshening in each month, enough observations were made so that the differences noted were highly significant. Standard errors of the difference between means were used in judging reliability. Comparing the average yield for Guernseys freshening in February with that for Guernseys freshening in July, the actual difference was about ten times the standard error of the difference between the means. This was very highly significant. Between two consecutive months differences were sometimes small and were not always significant. In general, however, average yields consistently increased from the least favorable to the most favorable month, and consistently decreased from the most favorable to the least favorable month.

Month of calving and milk yield—all breeds combined. The general patterns of the association of month of calving with milk yields were similar for each of the four breeds. Similarly, the general patterns were similar for each of the most important age groups, although the differences in yields between the most favorable and least favorable month of calving became relatively greater as age increased. These differences were sufficiently small, however, so that a single measure of the relationship of month of calving to milk yields was considered to be of value.

TABLE 5

Average milk yields, by breeds and for all breeds combined, for 22,212 cows freshening in different months

Month of freshening	Average milk yields*					No. of cases
	Guernseys	Holsteins	Ayrshires	Jerseys	All breeds combined	
	(lb. milk per cow, 305-day lactation period)					
Jan.	7540	9720	8520	7460	8450	1946
Feb.	7610	10120	8240	7230	8570	1766
March	7500	9790	8170	7250	8400	1874
April	7360	9770	7880	6890	8260	1630
May	7230	9390	7750	6420	8000	1492
June	6800	9200	7790	6570	7770	1572
July	6670	8930	7330	6260	7540	1876
Aug.	6950	8810	7620	6340	7650	2002
Sept.	6950	9220	7890	6630	7860	2046
Oct.	7380	9540	8070	6900	8210	2114
Nov.	7520	9650	8260	7010	8340	1990
Dec.	7550	9700	8280	6940	8360	1904
Av.	7260	9490	7980	6820	8120	22212

* Yields for each breed were calculated as described in tables 1 and 3. Yields for all breeds combined were calculated by weighting yields for each breed by the total number of observations, *i.e.*, 9,325 Guernseys, 8,260 Holsteins, 2,344 Ayrshires, and 2,283 Jerseys.

Average yields were highest for cows calving in February and lowest for cows calving in July (table 5). The difference in yields was 1,030 lb. Cows freshening in February produced 13.7 per cent more milk than those freshening in July. Yields increased from one month to the next from July to February, and decreased from one month to the next from February to July.[2]

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[2] In applying the relationships found in this analysis to individual farms, it must be kept in mind that they are for the average cow in dairy herd improvement associations in Connecticut. Feeding rates, pasture conditions, milk-producing capacity of cows, etc., might vary from the D.H.I.A. situation on any particular farm, and thus affect the relationship of month of calving to milk yields.

DISCUSSION

Woodward (2) studied the influence of the month of calving on milk production of 15,442 cows in dairy herd improvement associations in 12 states. The lactation records covered a 365-day period, rather than the 305-day period for the records used in the Connecticut analysis. Milk yields were corrected to a 4-per cent milk-equivalent basis. This was not done in the Connecticut study, since the price differential for butterfat places primary importance on milk yield rather than butterfat test.

A comparison of the association of month of calving with milk yields as found in the two studies is shown in figure 1. Milk yields for cows calving in the most favorable month in Woodward's study were only 2.5 per cent higher than for cows calving in the least favorable month. In the Connecticut analysis, milk yields in the most favorable month exceeded those in the least favorable month by 13.7 per cent. The range between yields in the low and high months was more than four times greater with Connecticut records than with the 12-state records used by Woodward. In absolute terms, milk yields in the most favorable month exceeded those in the least favorable month by 222 lb. in Woodward's study and by 1,030 lb. in the Connecticut study. The relatively small influence of month of calving on milk yields found by Woodward may have been due to his combining all records—all breeds, all ages, all states—before calculating average yields. If climatic differences should cause the association of month of calving with milk yields to vary among the different states, a combination of records from

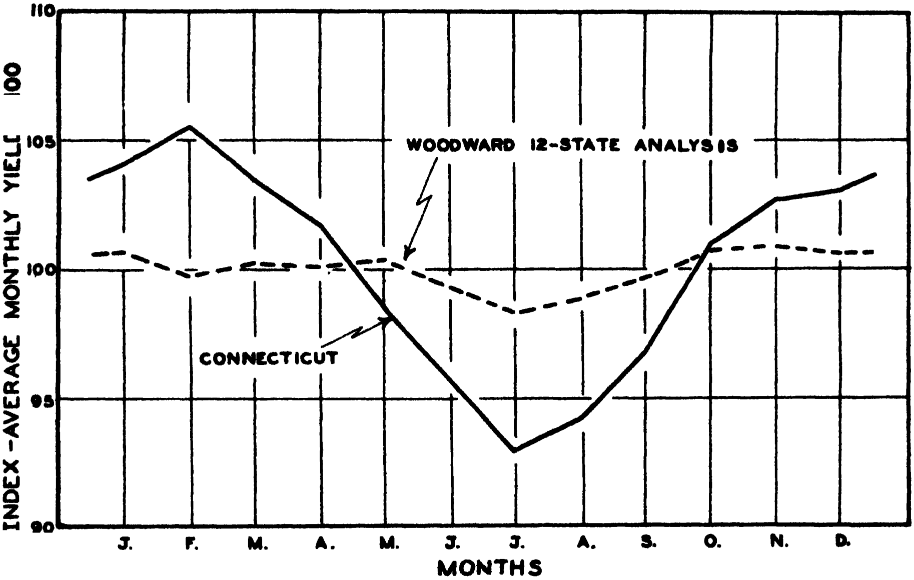

FIG. 1. Milk yields for cows calving in different months, 22,212 Connecticut lactation records compared with 15,442 12-state records analyzed by Woodward.

several states would tend to minimize fluctuations in yields. Greater variability might have been noted in Woodward's analysis if the effect of the joint relationship of age with season of freshening had been eliminated.

Although in both studies the month of July was found to be the least favorable month of calving, the most favorable month in the Connecticut study came in February rather than in November, as in Woodward's study. The reasons for this difference are not apparent.

In a recent New Hampshire study, Morrow *et al.* (1) reported average milk yields by breeds for 4,030 cows calving in different months. Production was calculated on the basis of a 305-day lactation period, and corrected to a 4-per cent milk-equivalent, mature-equivalent volume.

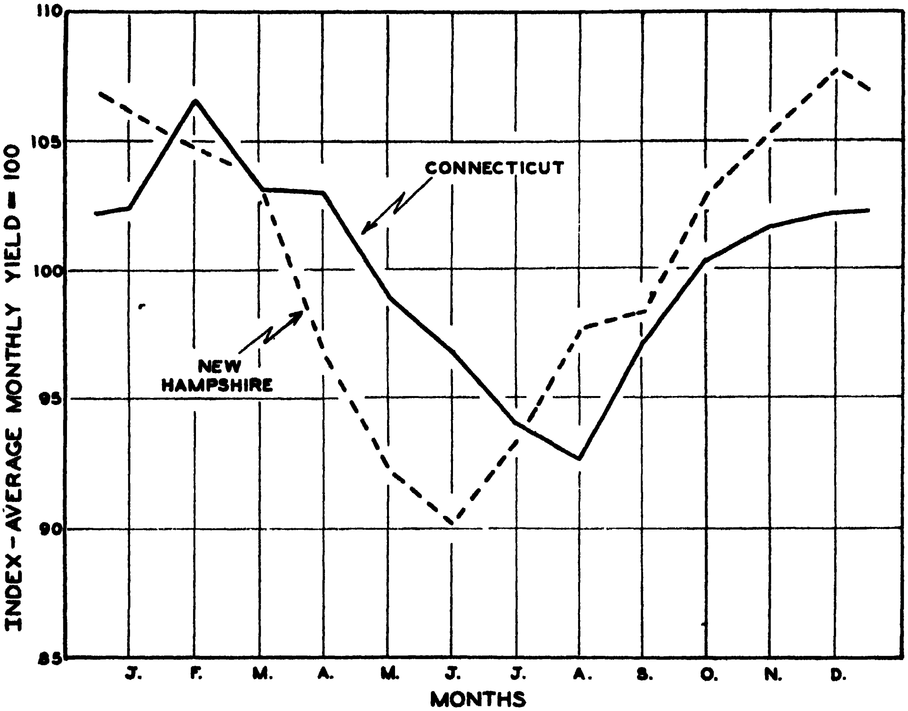

FIG. 2. Milk yields for Holstein cows calving in different months, 1,946 New Hampshire lactation records compared with 8,260 Connecticut records.

As shown in figure 2, the most favorable month of freshening for Holstein cows, according to the New Hampshire analysis, was December, and the least favorable was June. Average milk yields for cows freshening in December exceeded those freshening in June by 19.2 per cent. This variation was greater than that found in Connecticut, where the corresponding difference was 14.9 per cent. The most favorable month in Connecticut was February, and the least favorable month was August.

As shown in figure 3, Guernsey cows calving in January gave the most

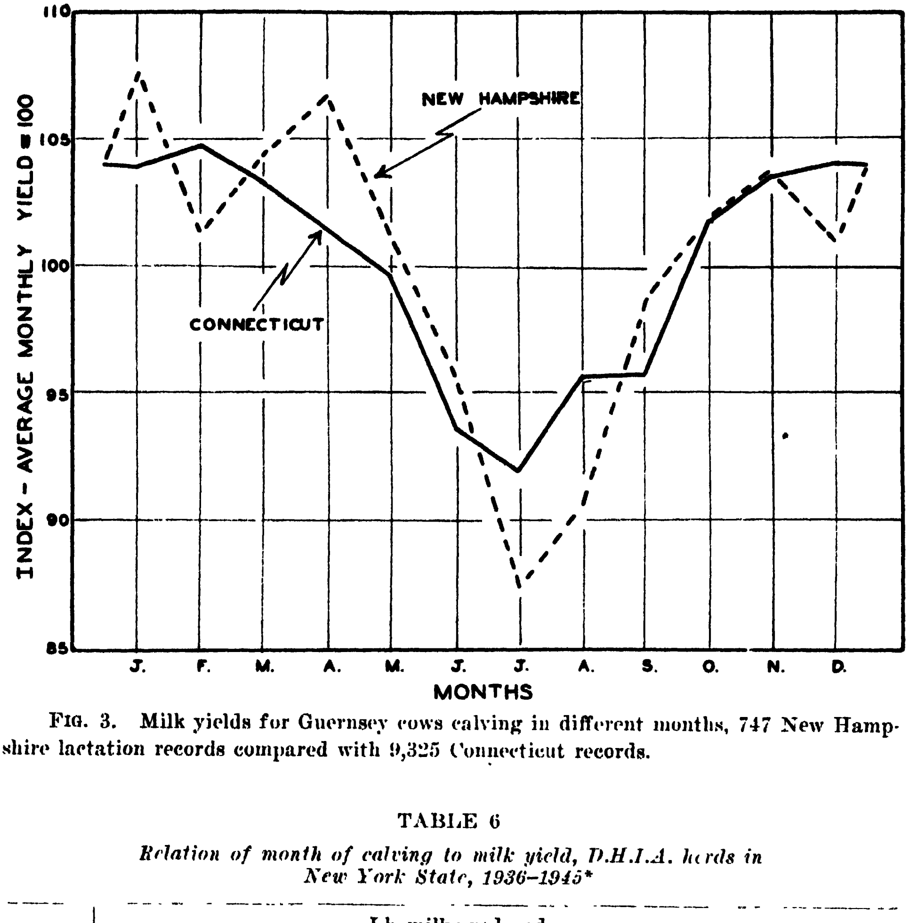

FIG. 3. Milk yields for Guernsey cows calving in different months, 747 New Hampshire lactation records compared with 9,325 Connecticut records.

TABLE 6

*Relation of month of calving to milk yield, D.H.I.A. herds in New York State, 1936–1945**

Month of freshening	Lb. milk produced per cow										
	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	10-yr. average
Jan.	8519	8247	8423	8410	8636	8830	9302	9482	9436	9598	8888
Feb.	7897	8047	8150	8032	8248	8606	8939	9245	9045	8895	8510
March	7752	7862	8078	7980	8265	8239	9141	9138	8736	8888	8408
April	7676	7786	7614	7781	8027	8108	8467	8603	8743	8661	8147
May	7721	7542	7666	7463	7847	8122	8473	8690	8430	8679	8063
June	7852	7952	7427	7465	7877	7712	8442	8500	8654	7999	7988
July	8085	7475	7838	7389	7950	8028	8334	8681	8292	8506	8058
Aug.	8542	8617	8059	7910	8047	8490	8695	8891	8614	8810	8468
Sept.	8590	8217	8341	8418	8434	8889	9177	9103	9262	9223	8765
Oct.	8687	8901	8833	8502	8429	8972	9391	9684	9380	9532	9031
Nov.	8676	8797	8958	8492	8768	9247	9383	9926	9471	9552	9127
Dec.	8116	8573	8432	8344	8680	9045	9309	9538	9199	9475	8871

*** Annual data obtained from J. F. Kendrick, Bureau of Dairy Industry, Washington, D. C., and from W. T. Crandall, Department of Animal Husbandry, Cornell University, Ithaca, New York. The 1936–1945 averages were computed by the authors of this report.**

milk, according to the New Hampshire analysis, and those in July gave the least. The low month was the same as in the Connecticut analysis, but the high month was 1 month earlier. Production in the most favorable month exceeded that in the least favorable month by 23.1 per cent in New Hampshire and 14.1 per cent in Connecticut.

A summary based on records of Dairy Herd Improvement Associations in New York State indicated that cows freshening in November gave the most milk and cows freshening in June gave the least milk (table 6). The least favorable month came 1 month earlier than in Connecticut, and the most favorable month 3 months earlier. The difference in yield between the most favorable and the least favorable month was about the same as in Connecticut.

SUMMARY

Milk yields for 22,212 Connecticut cows averaged highest for cows freshening in February and lowest for cows freshening in July. The most favorable month of calving came later in the fall-winter season than had been found in studies in other areas.

Production for cows calving in the most favorable month of freshening exceeded that in the least favorable month by 13.7 per cent. This was much greater than found by Woodward in a 12-state analysis but less than was found in recent studies in New Hampshire, and about the same as found in New York.

The association of milk yield with month of calving was similar for each of the four breeds—Guernsey, Holstein, Ayrshire, and Jersey. The association also was similar for each of the most important age groups, although the difference in yields between the most favorable and least favorable month of calving became relatively greater as age increased.

In determining differences in yields associated with month of calving, statistical procedures taking into account the joint relationship of age with season of freshening were necessary. As the cows in Connecticut herds increased in age, a smaller proportion freshened in the fall months of the year.

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CHOLINE STUDIES WITH YOUNG DAIRY CALVES. II. EFFECT OF MILK AND OTHER SUPPLEMENTS ON BLOOD CHOLINE LEVELS[1]

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In a previous publication (11) the authors presented evidence which indicates that the choline content of colostrum is reflected in the choline content of the blood of the young calf. The following study is a report of a continuation of this work. The experiments were designed to show trends of choline levels of blood with increasing age, and to determine effects of milk and other supplements in the diet upon the blood choline levels of young calves.

EXPERIMENTAL

In these experiments the method of analysis for choline in blood was the same as that used previously (11).

Effect of removal of milk from the diet. Trial I included seven calves. These calves were kept in individual pens and allowed the amount of grain mixture they would clean up twice daily. The ingredients of this mixture are shown in table 1. Alfalfa hay was fed *ad libitum* after the first 2 weeks of age. Two calves received milk throughout the trial. For the other five calves the removal of milk was started at 4 weeks of age and was completed by the fifth week

Figure 1 shows the results of this trial. Blood choline values definitely were depressed by the removal of milk from the calves' diets. This decrease was obvious even by the end of the fifth week, when the removal of milk was complete.

Trial II was conducted to obtain more evidence of the blood choline decline incident with removal of milk and was designed similar to trial I. However, a different grain mixture was used, as shown in table 1. This mixture contained about 1 mg. of choline per gram. Vitamins A and D were fed by capsule to insure a daily intake of 10,000 I.U. of vitamin A and 400 units of vitamin D for the first week, after which the amounts were decreased 50 per cent. All the dams of these calves had been on pasture just prior to calving, and the milk given these calves was from Holstein cows receiving some pasture. The Holstein calves received 10 lb. of milk daily and the Jersey and Guernsey calves, 8 lb. Four calves were used as controls and received milk throughout the trial. Milk was removed from the diets of

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[1] Journal Paper no. 301 of the Purdue University Agricultural Experiment Station.

[2] Now at North Carolina State College of Agriculture, Raleigh.

TABLE 1

Composition of grain mixtures

	Trial I	Trial II
	(*lb.*)	(*lb.*)
Ground yellow corn	32	25
Rolled oats	25	28
Wheat bran	15	15
Linseed oil meal	10	10
Soybean oil		20
Commercial casein	10	
Alfalfa leaf meal	5	
Iodized salt	1	1
Steamed bone meal		1
Mineral mixture*	1	
Cod liver oil	1	

* Bone meal, 50%; limestone, 46.7%; ferric citrate, 3%; copper sulphate, 0.1%; manganese, 0.1%; and cobalt chloride, 0.1%.

11 calves at the fifth week of age. The results of this trial are shown in figure 2. The differences in blood choline levels of the two groups were not as great as were observed in trial I but were found to be statistically significant. If trial II had been terminated at 7 weeks, the results of the two trials would have been very similar. The calves were not as thrifty as those in trial I. The incidence of scours was greater and appeared to cause a definite lowering of blood choline. Calves which became unthrifty at one time or another, as evidenced by low weight gains or loss in weight, usually had low or fluctuating blood choline values. The comparison of the two groups,

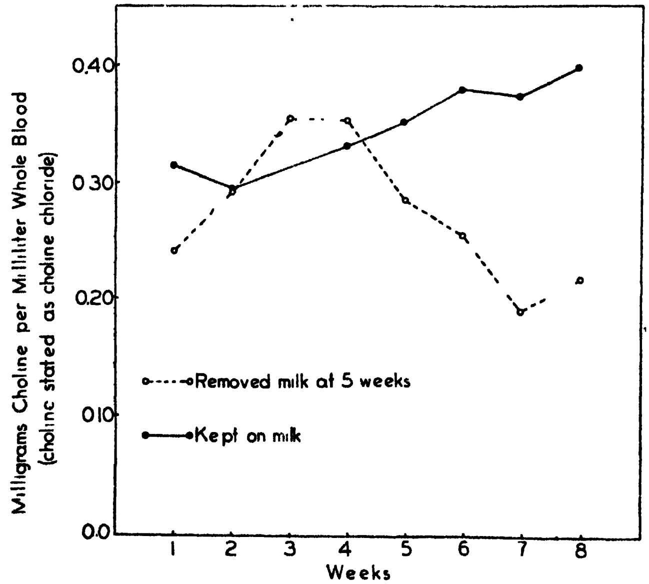

FIG. 1. The effect of milk consumption on the choline content of the blood of calves in trial I.

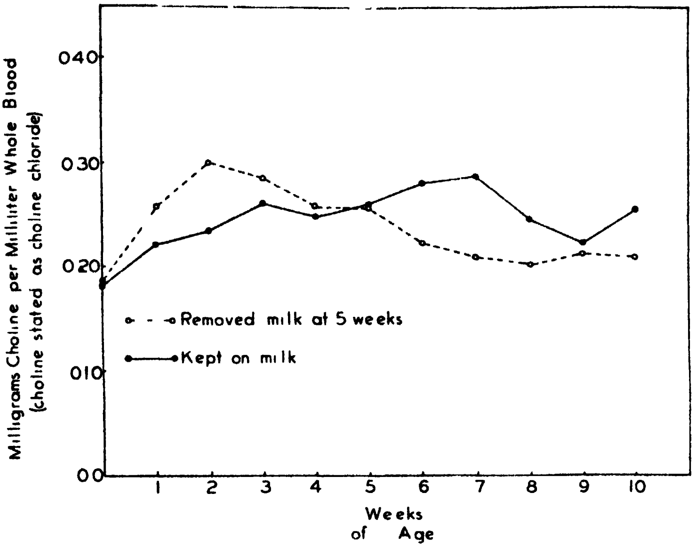

FIG. 2. The effect of milk consumption on the choline content of the blood of calves in trial II.

shown in figure 2, indicates that removal of milk from the calves' diets caused a lowering of blood choline.

Effect of feeding choline and methionine on blood choline. The removal of milk from the diet caused lower blood choline levels. Whether this depression of blood choline was due either to insufficient choline or to insufficient labile methyl groups (7, 8, 9, 10) was tested by feeding choline and methionine. Four calves which had not received milk after 5 weeks of age were used in this trial. For a period of 2 weeks, beginning at the eighth week of age, each calf was given by capsule a daily dosage of 12 g. of choline chloride, followed for 2 weeks by a daily dosage of 4 g. of choline chloride and 6 g. of dl-methionine.

The data of table 2 indicate that these supplements had no effect upon the

TABLE 2

Effect of feeding choline and methionine on blood choline levels
(mg. choline chloride per ml. whole blood)

	Age in weeks	JP317*	B587†	P124*	B588†	Av.
Initial	8	0.23	0.26	0.24	0.22	0.24
12 g. choline chloride daily	9	0.30	0.21	0.19	0.23	0.23
	10	0.24	0.16	0.25	0.21	0.22
4 g. choline chloride and 6 g. methionine daily	11	0.27	0.22	0.24	0.15	0.22
	12	0.26	0.22	0.22	0.19	0.22

* Jersey.
† Holstein.

level of blood choline. The weekly variations of blood choline values were not statistically significant. An insufficient quantity of choline or of labile methyl groups does not appear to be responsible for lowered blood values which occurred after the removal of milk from the diet.

Effect on blood choline of returning milk to the diet. A test was made to determine the effectiveness of the addition of milk to diets with and without choline supplements in raising blood choline values. Milk was restored to the diets of the same four calves which had received the choline and methionine supplements. The milk was increased gradually over a period of 4 days, after which the Jersey calves received 8 lb. of milk a day and the Holsteins, 10 lb. In addition to the milk, two of these calves were given 8 g. of choline chloride daily. As controls, four other calves of the same age received no milk. This trial began when the calves were 13 weeks of age and continued for 4 weeks. The results are given in table 3. The data on these small numbers should not be considered conclusive but it appears that the milk (and milk and choline) caused an elevation of blood choline when introduced into the diet of these calves which had received no milk for 7 weeks. The supplementation of the milk with choline chloride appeared to be without effect.

TABLE 3

Effect of restoring milk to the diet on the choline content of blood (mg. choline chloride per ml. whole blood)

Weeks	Control—no milk or choline					Milk		Milk & choline		Av.
	P125*	80B†	B589†	B582‡	Av.	JP317*	P124*	B587†	B588†	
Initial	0.20	0.24	0.27	0.24	0.24	0.26	0.22	0.22	0.19	0.22
1	0.24	0.22	0.26	0.21	0.23	0.30	0.28	0.25	0.23	0.27
2	0.26	0.27	0.26	0.26	0.26	0.29	0.28	0.29	0.28	0.29
3	0.22	0.21	0.22	0.19	0.21	0.30	0.35	0.25	0.22	0.28
4	0.23	0.15	0.22	0.22	0.21	0.31	0.28	0.31	0.30	0.30

* Jersey.
† Holstein.
‡ Guernsey.

Attempts to increase the blood choline by injections of choline chloride. Since oral administration of choline chloride did not appear to increase the choline content of the blood, possibly either the choline was not absorbed or its accumulation in the blood stream was prevented by rapid removal. In the previous experiments, the blood samples were taken approximately 24 hours after giving the choline. Therefore, even if the intestinal absorption of the choline were rapid and the subsequent removal from the blood were rather slow, any initial rise in the choline content of the blood following feeding the choline chloride might not have been detected. However, with the introduction of choline chloride directly into the blood stream, it was thought that an elevation of the choline level should be detected unless the removal from the blood is extremely rapid.

Choline chloride solutions were injected into one jugular vein, and blood samples were taken from the opposite jugular for choline analysis. The injected solution contained 1 g. of choline chloride per 4 ml. of water, and injections were made at the rate of about 1 g. of choline chloride per minute. The injection of 1 g. of choline chloride into an 80-lb. calf should be sufficient to approximately double the choline content of the blood.

The blood choline values obtained just before and at intervals following the injections are shown in table 4. The first injection was made into calf no. 79B, a Holstein heifer. This calf lacked thrift and had made slow weight gains, but an autopsy at a later date showed no pathological symptoms. This calf became prostrate within 60 seconds after the injection and showed difficulty of breathing, considerable salivation, some belching of gas, and defecation and urination within the first 3 minutes. When the calf was helped to its feet after 5 minutes, it was very unstable, with considerable quivering of the muscles. The calf appeared normal 15 minutes after injection. These reactions were typical of all injections except that they did not always cause complete collapse.

TABLE 4

Effect of injecting choline chloride on blood choline levels
(mg. choline chloride per ml. whole blood)

Calf	80B*	79B*	79B	79B	B591†	B591	B591
Date (1946)	Nov. 11	Nov. 4	Nov. 11	Nov. 19	Nov. 11	Dec. 2	Dec. 17
G. choline injected	0	1	1	2	1	1	2
Calf weights (lb.)	165	80	82	84	80	97	107
Before	0.24	0.23	0.14	0.16	0.22	0.24	0.14
3 min.‡	0.25		0.24	0.50	0.25	0.22	0.30
6 "		0.28	0.23		0.21	0.16	0.16
10 "	0.24			0.32			Dead
15 "	0.20	0.18	0.13	Dead	0.20	0.18	
30 "	0.21		0.17		0.21	0.17	
60 "	0.23	0.20	0.18		0.17	0.15	
240 "	0.25	0.20			0.22	0.20	

* Holstein.
† Guernsey.
‡ Minutes following beginning of injection.

The increases following 1 g.-injections were not consistent. The 2 g.-injections produced a definite increase in the choline of the blood although this amount proved fatal. It appears that the injected choline was removed quickly from the blood stream. To check the possibility that handling and repeated bleeding might influence the choline levels, calf no. 80B was bled at intervals without injection. These effects were insignificant.

DISCUSSION

It might appear that there is a simple correlation between the choline intake with milk and the choline level of the blood. As shown in previous work (11), the choline content of the blood increased considerably when the calves received colostrum, which is high in choline. This response might be analogous to the response of vitamin A in the calves' blood due to the high vitamin A content of colostrum. In this study the choline content of blood remained high until the withdrawal of milk, when it decreased. The choline content also appeared to be elevated by returning milk to the diet.

That these responses are not directly related to the choline of the diet is indicated by two observations. In the first place, when the milk was removed from the diets, the calves consumed about 1 lb. more grain per day. The choline content of this additional grain (0.45 g.) largely compensated for the loss of choline due to the removal of milk (0.63 g.). In the second place, administration of additional choline and methionine with the grain ration failed to increase blood choline levels. Consequently, the drop in blood choline hardly can be attributed to the lack of choline or transferable methyl groups, since the dietary intake of choline cannot be correlated with the blood choline levels.

Since there does not appear to be a simple quantitative relation between choline intake and blood levels, it might appear that the choline of the grain mixture was less available than that in the milk. Some work with poultry (5) indicates that the choline of some grain mixtures is not utilized as well as in others. The supplementation of the grain mixture with comparatively large amounts of choline chloride would be expected to cause increases in blood choline, even though utilization of the added choline was very inefficient.

Inasmuch as milk favored higher choline levels than grain, possibly milk contains choline precursors. Studies with chickens indicate that poultry are capable of some choline synthesis and that some feeds contain choline precursors (4, 6). However, the supplementation of the grain with choline chloride and methionine should have compensated for any precursors of choline of the milk diet.

Inasmuch as choline is present as lecithin in both grains and milk, this would not explain the differences. However, Artom and Cornatzer (1) recently observed that in rats more synthesis of phospholipids occurred in the intestinal walls when fat and choline were ingested than when choline or fat alone was ingested. Fishman and Artom (3) also have shown that liver lecithin is greater when rats are fed high fat diets than when fed low fat diets, if adequate choline is present. The calves, while receiving milk, did have considerably more fat in their diets than when milk was removed. It may be that the greater amount of fat necessitated more phospholipid formation for efficient utilization of the fat, and the higher amounts of choline occurred in the blood because of the type of nutrient being transported. On the basis of these observations (1, 3), it seems plausible that the differences in fat content of the two diets might be related to differences found in blood choline levels.

In consideration of the evidence previously presented, it appears that some component of milk other than choline or methionine may influence blood choline levels of young calves.

Choline chloride is toxic when injected intravenously. This is in considerable contrast to the apparently low toxicity when fed orally. No deleterious effects were noted when as much as 12 g. of choline chloride per day was given orally, but it may be that prolonged feeding of these rather large amounts would produce undesirable results. The injected choline chloride was removed from the blood stream very rapidly. The lethal amount of choline chloride for 80- to 100-lb. calves when injected at a rate of about 1 g. per minute appeared to be between 1 and 2 g.

SUMMARY

1. Removal of milk from the diets of calves at 5 weeks of age resulted in a drop in blood choline values.

2. Feeding choline chloride or choline chloride and methionine did not increase blood choline levels.

3. While not conclusive, some data are presented that indicate returning milk to the diet of calves caused elevation of blood choline.

4. Evidence is presented which indicates that a component of milk other than choline or methionine may influence blood choline levels.

5. Intravenous injections of choline chloride produced only a temporary rise of the choline content of the blood. It was rapidly removed from the blood stream. Injections of 2 g. proved to be fatal.

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THE PHARMACODYNAMICS OF ASCORBIC ACID IN CATTLE. I. ABSORPTION AND ELIMINATION[1, 2]

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It has been believed generally that cattle synthesize sufficient quantities of ascorbic acid to meet their requirements. Several investigators (20, 30, 33, 34) fed dairy cattle for extended periods on diets low in ascorbic acid without observing significant decreases in blood plasma ascorbic acid or decreased excretion of the vitamin in milk or urine. Others (9, 21, 22, 32) have reported that oral administration of crystalline ascorbic acid in large quantities did not increase the ascorbic acid content of the milk. Whitnah and Riddell (35) and Riddell *et al.* (22) fed 65 kg. of green rye to dairy cows which were otherwise kept under barn feeding conditions. They observed several-fold increase in the urinary excretion of ascorbic acid and a doubling of the blood plasma levels during the first 12 hours. The blood plasma and urinary ascorbic acid returned to pre-feeding levels within 24 hours. No significant changes occurred in the milk ascorbic acid as a result of feeding green rye.

Rasmussen *et al.* (19) and Knight *et al.* (9) have shown that intravenous or subcutaneous administration of crystalline ascorbic acid results in significant increases in this compound in cows' milk. Investigations of rumen contents by Whitnah and Riddell (35), Knight *et al.* (10), and Vavich *et al.* (32), both *in vivo* and *in vitro,* have shown that ascorbic acid is lost rapidly. Crystalline ascorbic acid was destroyed more rapidly than that from plant sources, probably because of the slower release from the plant tissues; Vavich *et al.* (32) showed that ascorbic acid destruction was decreased but not entirely prevented in autoclaved rumen contents.

The probable synthesis of ascorbic acid by the dairy cow has been suggested (8, 10, 11, 20, 22, 31, 33, 34), but the site of synthesis is unknown.

It generally is believed that blood plasma ascorbic acid is increased most effectively when vitamin C is administered subcutaneously or intravenously. Hawley *et al.* (7), Knight *et al.* (10), and Satterfield *et al.* (24) have revealed that blood levels rise very rapidly following intravenous administration, with excretion in the urine practically being completed within 2 hours.

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Subcutaneous administration results in a slower rise in blood levels, with a correspondingly slower rate of excretion. Oral administration of chlorobutanol (2, 3, 14, 15, 16, 17, 26) significantly increases blood plasma ascorbic acid in cattle and urinary excretion of ascorbic acid is increased greatly (16, 17). This phenomenon also has been demonstrated in rats (12, 28). Feng (6) reported that chlorobutanol and phenobarbitol accelerate the urinary excretion of ascorbic acid by rats and increase its concentration in small intestinal tissue, kidney, spleen, and liver.

Although the bovine apparently does not require extraneous ascorbic acid in the diet, considerable quantities of the vitamin are ingested, especially when animals are on good pasture. In view of the controversial nature of ascorbic acid utilization and its indicated destruction in the rumen, the study of absorption and elimination of ascorbic acid when administered into various known levels of the digestive tract, as compared to subcutaneous and intravenous administration, seemed highly desirable. Since chlorobutanol previously had been shown to increase blood plasma ascorbic acid, it seemed advisable to study its effects.

EXPERIMENTAL

Steers were selected for these studies because of the ease of continuous urine collection and the absence of such disturbing side effects as estrus and lactation. Five yearling Hereford cattle averaging 600 lb. at the beginning of the experiment were used. They received daily 6 lb. of a grain mixture composed of four parts shelled corn, two parts whole oats, and one part soybean meal and were fed good quality alfalfa hay *ad libitum*. Feeding and watering were done between 7:00 and 8:00 a.m. and 5:30 and 6:00 p.m. The steers were kept in metabolism stalls for several days before and following each experimental trial and in a small dry lot at other times. Rumen fistulae were made in two steers using the technique of Schalk and Amadon (25), and fistulae were created in the jejunum, cecum, and colon, respectively, in the other three animals, as described by Nichols (18). Twenty-four-hour urine samples were collected during the experimental trials on all steers except the steer with the small intestinal fistula. The abdominal location of this fistula made urine collection impractical. Ascorbic acid determinations were made on whole blood, blood plasma, urine, feces, and ingesta, using the method of Roe and Keuther (23). Hemoglobin was determined by the method of Evelyn (5) and blood cell volume by the method of Wintrobe (36). All colorimetric determinations were made with the Evelyn photoelectric colorimeter.

Twenty-four-hour urine samples were collected before trials, during trials, and 1 to 2 days afterwards. The urine was collected in 5-l. bottles charged uniformly with 100 c.c. of 20 per cent metaphosphoric acid. Urine specimens were measured and sampled uniformly between 7:30 and 8:00

a.m. and 5:30 and 6:00 p.m. All statistical analyses were made according to Snedecor (27).

RESULTS

Control. Since preliminary trials indicated that blood plasma ascorbic acid was decreased by frequent bleeding, this factor was investigated. Two trials on each of four steers (those with rumen, colon, and cecum fistulae) were made. Base levels of urinary excretion of ascorbic acid and blood content of the vitamin were established. The animals then were injected with 50 c.c. of distilled water into their various fistulae and were bled ten times during an 8-hour period. The results, as shown in table 1, reveal that whole blood and blood plasma ascorbic acid levels were significantly decreased, as compared to base levels (mean of – 24, 0, and 24 hours used uniformly in this and succeeding trials discussed in this section), during the period when bleeding times were close together. These levels returned to pre-trial levels between the fourth and sixth hours. The extreme drop in blood plasma ascorbic acid for the fourth hour is not readily explainable, but in six of the eight trials this value was lower than for the third hour and in seven trials it was lower than for the sixth hour. The urine excretion for this period (table 2) shows little variation for the 3-day periods involved. The variance between steers, however, was highly significant.

Rumen. Six trials were made on each of two steers. Sixteen grams of ascorbic acid, dissolved in 100 c.c. of distilled water, were delivered onto the floor of the ventral rumen sac with a syringe and catheter. This procedure was repeated every other day for three trials; the steers then were given a 13-day rest period before the second series of three trials was run. As shown in table 1, significant increases in whole blood and blood plasma ascorbic acid were observed 15 and 30 minutes after placing the vitamin in the rumen. Whole blood ascorbic acid was increased over base levels for the first 4 post-injection hours, but blood plasma values dropped noticeably after 1.5 hours.

Inspection of the data for individual steers reveals that in eleven of twelve trials both whole blood and blood plasma ascorbic acid were increased; the maximum response occurred 15 minutes after injection in ten trials. In some trials increased levels were maintained for 4 hours after treatment, but variation in the duration of response and the tendency for repeated bleeding to lower blood ascorbic acid made average increases over base levels after 30 minutes rather small. In table 2 the urine response is recorded for each steer and for both rumen steers. The steers with rumen fistulae showed approximately the same increase in urinary elimination on the day of treatment as compared to the day before and the day after. The variance between days and between steers for the two rumen steers analyzed together was significant ($P < 0.05$), while the variance between trials × steers was nonsignificant.

TABLE 1

Effects of administration of ascorbic acid into the digestive tract on blood ascorbic acid

Administration of ascorbic acid		No. of sample series	Interval of bleeding[a]	Ascorbic acid			
				Whole blood		Blood plasma	
					Diff.[b]		Diff.[b]
(g.)	*(site)*			*(mg.%)*	*(mg.%)*	*(mg.%)*	*(mg.%)*
0	Control[c] series	8	−24 hr.	0.418		0.270	
			0	0.410		0.282	
			15 min.	0.395	−0.021	0.316	0.027
			30 min.	0.391	−0.025	0.300	0.011
			1 hr.	0.352	−0.064[d]	0.269	−0.020
			1½ hr.	0.340	−0.076[d]	0.250	−0.039
			2 hr.	0.386	−0.030	0.295	0.006
			3 hr.	0.395	−0.021	0.294	0.005
			4 hr.	0.372	−0.044[e]	0.200	−0.089[d]
			6 hr.	0.446	0.030	0.356	0.067[e]
			8 hr.	0.445	0.029	0.301	0.012
			24 hr.	0.419		0.315	
16	Floor ventral rumen sac	12	−24 hr.	0.568		0.474	
			0	0.603		0.492	
			15 min.	0.698	0.110[d]	0.598	0.113[d]
			30 min.	0.667	0.079[d]	0.548	0.063[e]
			1 hr.	0.634	0.046	0.517	0.032
			1½ hr.	0.625	0.037	0.528	0.043
			2 hr.	0.638	0.050	0.467	−0.018
			3 hr.	0.614	0.026	0.503	0.018
			4 hr.	0.639	0.051[e]	0.478	−0.007
			6 hr.	0.587	−0.001	0.469	−0.016
			24 hr.	0.592		0.488	
4	Cecum	10	−24 hr.	0.455		0.370	
			0	0.519		0.389	
			15 min.	0.624	0.133[d]	0.527	0.147[d]
			30 min.	0.561	0.070	0.479	0.099[d]
			1 hr.	0.587	0.096[e]	0.447	0.067[e]
			1½ hr.	0.555	0.064	0.439	0.059
			2 hr.	0.570	0.079	0.431	0.051
			3 hr.	0.556	0.065	0.459	0.079[e]
			4 hr.	0.528	0.037	0.426	0.046
			6 hr.	0.508	0.017	0.390	0.010
			24 hr.	0.498		0.381	
4	Colon	10	−24 hr.	0.457		0.362	
			0	0.525		0.410	
			15 min.	0.596	0.104[d]	0.491	0.115[d]
			30 min.	0.548	0.056	0.449	0.039
			1 hr.	0.575	0.083[e]	0.446	0.036
			1½ hr.	0.591	0.099[e]	0.478	0.068
			2 hr.	0.601	0.109[d]	0.449	0.039
			3 hr.	0.536	0.044	0.439	0.029
			4 hr.	0.500	0.008	0.340	−0.036
			6 hr.	0.481	−0.011	0.370	−0.006
			24 hr.	0.494		0.356	

TABLE 1 (*Continued*)

Administration of ascorbic acid		No. of sample series	Interval of bleeding[a]	Ascorbic acid			
				Whole blood		Blood plasma	
					Diff.[b]		Diff.[b]
(*g.*)	(*site*)			(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)
Summary	Rumen, cecum, colon	32	−24 hr.	0.498		0.407	
			0	0.552		0.434	
			15 min.	0.643	0.116[d]	0.540	0.122[d]
			30 min.	0.597	0.070[d]	0.495	0.077[d]
			1 hr.	0.601	0.074[d]	0.473	0.055[d]
			1½ hr.	0.591	0.064[d]	0.485	0.067[d]
			2 hr.	0.605	0.078[d]	0.450	0.032[e]
			3 hr.	0.571	0.044[e]	0.469	0.049[d]
			4 hr.	0.561	0.034	0.419	0.001
			6 hr.	0.529	0.002	0.413	−0.005
			24 hr.	0.532		0.413	
4	Small intestine (jejunum)	8	−24 hr.	0.319		0.176	
			0	0.328		0.226	
			15 min.	0.442	0.115[e]	0.355	0.150[d]
			30 min.	0.525	0.198[d]	0.388	0.183[d]
			1 hr.	0.426	0.099[e]	0.360	0.155[d]
			1½ hr.	0.366	0.039	0.276	0.071
			2 hr.	0.372	0.045	0.290	0.085
			3 hr.	0.415	0.088[e]	0.288	0.083
			4 hr.	0.356	0.029	0.269	0.064
			6 hr.	0.389	0.062	0.309	0.104[e]
			24 hr.	0.335		0.214	

[a] F-ratios between intervals were highly significant for each type of experiment reported below.

[b] Interval means compared to mean of −24, 0, and 24 hr.

[c] Distilled water injected into rumen, cecum and colon—4 steers, 2 trials each.

[d] Highly significant mean difference.

[e] Significant mean difference.

TABLE 2

Effects of administration of ascorbic acid into the digestive tract on urinary ascorbic acid

Administration of ascorbic acid		No. of 24-hr. samples in each period	Av. daily excretion of ascorbic acid in urine		
			Day before	Day of treatment	Day after
(*g.*)	(*site*)		(*mg.*)	(*mg.*)	(*mg.*)
0	Control	8	34.3	40.9	36.5
16	Rumen—1	6	90.6	123.5	75.1
16	Rumen—2	6	58.8	89.0	56.8
Rumen summary		12	74.7	106.3	65.9
4	Cecum	10	46.0	47.4	42.4
4	Colon	10	65.8	72.0	57.6
Rumen, cecum, and colon summary[a, b]		32	63.0	77.2	56.0

[a] Least significant mean difference between days is ±9.6 mg. of ascorbic acid; ±12.8 mg. required for a highly significant mean difference.

[b] Variance between days, between trials, and between steers was highly significant.

Cecum. Ten trials were made on one steer following the same general procedure previously described. Four grams of ascorbic acid in 25 c.c. of distilled water were administered into the cecum with a stomach tube. The average maximum increase in blood ascorbic acid occurred 15 minutes after treatment (table 1); both whole blood and plasma showed substantial increases for the first 3 hours, with a gradual decline to base levels thereafter. In some trials the maximum response occurred as late as 3 hours. Variation in the exact site of administration probably is responsible for the variation in time of maximum increase. Urinary excretion of ascorbic acid (table 2) was not significantly increased during the 24-hour period after administration of ascorbic acid, and the between-trial variance was not significant.

Colon. Four grams of ascorbic acid were administered in 25 c.c. of distilled water during each of ten trials on one steer. One-half of the ascorbic acid solution was injected approximately 18 inches anterior to the fistula and the remainder approximately 14 inches from the opening. As shown in table 1, maximum response in blood ascorbic acid again occurred within 15 minutes after the administration of the vitamin. Whole blood ascorbic acid levels were significantly higher for the first 2 hours, while blood plasma values dropped noticeably after 15 minutes but remained above base levels for the first 3 hours. Positive responses occurred in eleven of twelve trials for whole blood and in ten of twelve trials for blood plasma. The maximum response was delayed in three trials but occurred within the first 2 hours in every case where responses were noted. As shown in table 2, a slight increase in urinary excretion of ascorbic acid was observed for the day of treatment, but the variance between days and between trials was non-significant.

Rumen, cecum, and colon summary. Since administration of ascorbic acid into various levels of the digestive tract revealed approximately the same type of response in blood ascorbic acid, these data were analyzed collectively to increase the total number of replications to 32. The results, as shown in table 1, indicate that increases in blood ascorbic acid could not be detected 4 hours after administration of the vitamin. The observed effects may have been due to the level of ascorbic acid dosage. While four times more ascorbic acid was used in the rumen studies, the amount appearing in the blood at any one time was no greater than when 4 g. were used in the cecum and colon studies. This does not necessarily indicate there was only one-fourth the absorption, since significant increases were observed in urinary elimination of ascorbic acid in the case of the rumen studies, while only slight increases were observed in the colon and cecum studies. A second point to consider is the base level of ascorbic acid in whole blood and blood plasma. The base levels of the four animals throughout these studies frequently were higher than one normally would expect, thus making it less likely that more ascorbic acid would be detected in venous blood over a very long period of time after ascorbic acid was administered.

The urinary excretion of ascorbic acid also was analyzed collectively (table 2). The increased number of replications used in the analysis of variance reveals that highly significant differences occurred between days, between steers, and between trials × steers.

Small intestine. Eight trials were made on a steer with a fistula in the jejunal area of the small intestine. The same bleeding routine was practiced, but no urine specimens could be collected because of leakage from the intestine, which frequently ran down the sheath. Four grams of ascorbic acid in 25 c.c. of distilled water were administered with a stomach tube approximately 24 inches into the intestine. A catheter was fitted with a rubber thumb stall and the fistula opening closed immediately after the ascorbic acid was injected. This device was left in place for the first 90 minutes. This steer later died from intussusception of the intestine at the site of the fistula.

As shown in table 1, maximum response in blood ascorbic acid occurred 30 minutes after administration and persisted at a high level for the first hour. This steer showed a much lower base level of ascorbic acid than the other steers in these experiments. This may account for the 15-minute delay in average maximum response as compared to the other steers. In only two trials out of eight was the maximum blood response noted at 15 minutes, but the other six maxima occurred within the first 90 minutes. The degree of response also was quite variable. In two trials the blood levels were more than doubled while in two others the response hardly was detectable.

Further rumen absorption studies. Since adding ascorbic acid directly onto the rumen floor gave positive evidence of absorption, it seemed desirable to administer ascorbic acid into the intact rumen. The two steers with fistulae in the cecum and colon were used for this study. Sixteen grams of ascorbic acid in 100 c.c. of distilled water were administered for 3 successive days by stomach tube. Blood samples were drawn at 0, 15, and 30 minutes, and at 1, 2, 4, 5, and 24 hours for each trial, and urine samples were collected. The average whole blood and blood plasma ascorbic acid values were not significantly increased (fig. 1) over the base levels (average of 0 and 24 hours used as the base level). A definite positive response did occur in three of the six trials, but this response did not occur regularly at any one time interval. The steers eliminated on the average 82.2 mg. of ascorbic acid the day before and the day after treatment and 110.4 mg. daily during treatment. The magnitude of the increase was essentially the same as in previous trials with rumen fistulae. The variance between days was significant with the first day of treatment, showing increased urine excretion considerably above the day before, and 1 and 2 days after treatment. However, the number of trials is too small to say positively that this observed difference was real. Sixteen grams of ascorbic acid would be diluted considerably by the rumen contents and it would be difficult to select a bleeding interval that would show if and when maximum absorption occurred.

The administration of 16 g. of ascorbic acid 6 inches into the reticulo-omasal-abomasal orifice also was performed in four trials on the two steers

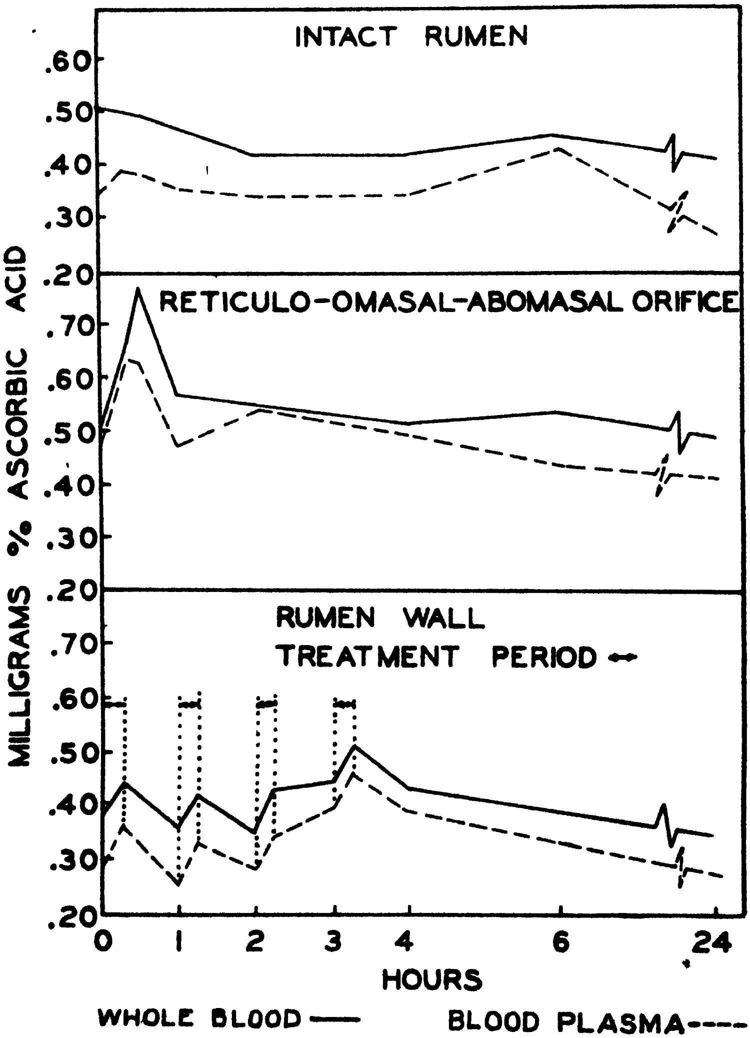

Fig. 1. The effect of administering ascorbic acid in various regions of the rumen on blood ascorbic acid.

with rumen fistulae. Base levels for blood and urine were established and the vitamin given on 2 successive days. The reticulo-omasal-abomasal orifice was located manually and a stomach tube inserted until air blown through it could not be felt bubbling back. Cold ascorbic acid solution

TABLE 3

Effects of administration of ascorbic acid directly into the reticulo-omasal-abomasal orifice

Steer	Urinary ascorbic acid				
	2 days before	1 day before	1st day treatment	2nd day treatment	Day after
	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)
Rumen—1	126.4	156.4	311.0	589.9	183.4
Rumen—2	96.0	23.7	214.2	209.5	137.9
Av.	111.2	90.0	262.6	399.7	160.6

then was passed through the tube. In none of the trials could the solution be felt oozing back into the rumen at the time of administration. The results (fig. 1) reveal that definite absorption occurred from this area. Whole blood ascorbic acid values reached a maximum in the first 30 minutes, while blood plasma values were highest at 15 minutes but remained at a high level through 30 minutes. These mean differences were highly significant. All of the trials showed a marked increase at either 15 or 30 minutes after administration for both whole blood and blood plasma ascorbic acid. Urinary elimination of ascorbic acid, as shown in table 3, was more than doubled the first day of treatment and tripled the second day. The response still was observable on the day following treatment.

Since placing ascorbic acid in an area where it by-passed the rumen gave such a marked positive response of absorption, it seemed highly desirable to see if ascorbic acid is absorbed when held in direct contact with the rumen wall. A petri dish was fitted with a piece of cellulose sponge to serve as the receptacle for ascorbic acid solution. This petri dish and sponge held 80 c.c. of solution and could be held in an upright position without loss of liquid. Six grams of ascorbic acid in 0.5 per cent sodium chloride solution were used for each of 24 trials on two steers. The procedure was to draw a blood sample, hold the solution against the upper ventral rumen wall for 15 minutes, and draw a second blood sample. This

TABLE 4

Holding ascorbic acid solution against the upper ventral rumen wall

Steer	Urinary elimination of ascorbic acid			
	1 day before	1st day treatment	2nd day treatment	Day after
	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)
Rumen—1	183.4	219.4		141.7
Rumen—1	120.4	143.1	115.7	36.4
Rumen—2	137.9	132.8	.	71.3
Rumen—2	90.5	82.7	42.6	27.4
Av.	133.0	144.5	79.2	69.2

procedure was repeated each hour on each steer for a total of 4 hours on 3 different days. The results, as shown in figure 1, reveal that ascorbic acid increased in both whole blood and plasma after 15 minutes. These differences were highly significant after 3.25 hours as compared to base levels (mean of – 24, 0, and 24 hours compared to other means). Urinary elimination, as shown in table 4, was not changed significantly.

Subcutaneous administration. For comparative purposes ascorbic acid was administered subcutaneously to the same four steers on which urine data from previous trials were available. Two grams of ascorbic acid were injected in the foreflank region of each steer and blood samples were drawn at 1, 2, 3, 4, 6, 8, and 24 hours post-injection. A total of eight trials were run. As shown in figure 2, peak levels of ascorbic acid in blood were reached within 3 hours after subcutaneous administration and returned to pre-treatment levels within 24 hours. However, all time interval means from 1 to 8 hours after administration were significantly higher than the base levels (mean of – 24, 0, and 24 hours afterwards). It should be noted that blood plasma ascorbic acid was higher than whole blood at the fourth hour, with the two being essentially the same at the third hour. Data on individual trials show that maximum blood ascorbic acid values occurred consistently between 3 and 6 hours after administration and blood plasma values were higher than whole blood in six of the eight trials at the fourth hour.

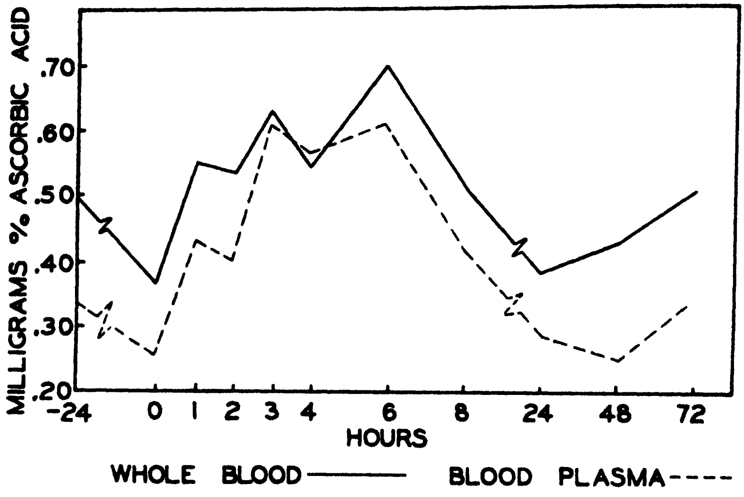

FIG. 2. The effect of subcutaneous ascorbic acid administration on blood ascorbic acid.

The amount of subcutaneously administered ascorbic acid recovered in the urine was surprisingly small. As shown in table 5, urinary excretion of ascorbic acid increased nearly six-fold but only 9.3 per cent of the administered vitamin was recovered as such in the urine. The range of recovery

TABLE 5

Ascorbic acid recovered in the urine following subcutaneous administration

Steer	No. of samples	Urinary ascorbic acid					Recovered in urine[a]
		2 days before	1 day before	Day of treatment	1 day after	2 days after	
		(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(%)
Colon	2	89.4	74.2	322.4	59.8	77.9	12.0
Cecum	2	34.7	29.2	183.2	70.2	86.2	7.6
Rumen—1	2	40.8	36.4	191.3	42.0	56.0	6.0
Rumen—2	2	32.8	23.4	131.2	58.2	33.8	5.2
Av.		49.4	40.8	232.0	58.8	63.5	9.3

[a] Mean of day of treatment—mean of 1 and 2 days before/2000 × 100; 2 g. of ascorbic acid were administered subcutaneously.

of ascorbic acid in individual trials varied from 1.5 to 32.5 per cent; both the highest and lowest recovery were observed on the same steer (colon).

Intravenous administration of ascorbic acid. To study speed of removal of ascorbic acid from the blood stream, 2 g. of ascorbic acid were given intravenously during eight trials on four steers. Blood samples were drawn 24 hours before, at 0, 1, 2, 4, 8, 16, 32, 60, and 90 minutes, and at 2, 3, 4, and 24 hours after intravenous administration of ascorbic acid. Urine samples were taken the day before and at 4, 12, 24, and 48 hours after treatment. The results are shown in tables 6 and 7. Intravenously administered ascorbic acid largely disappeared from the blood stream within 30 minutes, with small quantities being demonstrated in the blood for 4 hours.

TABLE 6

Rate of removal of intravenously administered ascorbic acid

No. of sample series	Time of bleeding	Av. ascorbic acid			
		Whole blood		Blood plasma	
			Diff.[a]		Diff.[a]
		(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)
8	−24 hr.	0.426		0.234	
	0	0.485		0.372	
	1 min.	5.05	4.60	6.11	5.82
	2 min.	3.94	3.49	4.60	4.31
	4 min.	2.86	2.41	3.42	3.13
	8 min.	2.20	1.75	2.40	2.11
	16 min.	1.22	0.77	1.54	1.25
	32 min.	0.749	0.299	0.782	0.496
	60 min.	0.538	0.088	0.529	0.243
	90 min.	0.516	0.066	0.460	0.174
	2 hr.	0.532	0.082	0.478	0.192
	3 hr.	0.546	0.096	0.452	0.166
	4 hr.	0.516	0.056[b]	0.469	0.183
	24 hr.	0.440		0.251	

[a] Mean of − 24, 0, and 24 hr. after.

[b] Not significantly different from base level.

Blood plasma ascorbic acid was considerably higher than whole blood ascorbic acid for the first 16 minutes and remained very near whole blood for the first hour. As shown in table 7, only 12.2 per cent of the ascorbic acid was recovered in the urine. The range of recovery in individual trials was 7.0 to 18.3 per cent. During the day of treatment 53 per cent of the ascorbic acid was eliminated in 4 hours, 33 per cent in the next 8 hours, and 14 per cent in the next 12 hours. This latter figure does not represent much more ascorbic acid than would have been eliminated in 12 hours if no ascorbic acid had been given.

Digestive tract administration of chlorobutanol. Base ascorbic acid levels on the four animals used in the intravenous study were established. Five grams of chlorobutanol then were given daily to each steer for 15 days.

TABLE 7

Ascorbic acid recovery in the urine following intravenous administration

Steer	No. of samples	Av. ascorbic acid in urine						
		Day before	Day of treatment	Day after	Recovery[a]	Day of treatment		
						1st 4 hr.	Next 8 hr.	Next 12 hr.
		(*mg.*)	(*mg.*)	(*mg.*)	(*%*)	(*mg.*)	(*mg.*)	(*mg.*)
Colon	2	61.8	317.4	86.6	12.8	130.6	110.8	75.9
Cecum	2	86.5	271.5	40.2	9.2	195.2	59.6	16.6
Rumen—1	2	73.0	389.7	53.4	15.8	190.2	136.6	63.0
Rumen—2	2	27.4	251.8	30.2	11.2	131.1	100.0	20.8
Av.	.	62.2	307.6	52.6	12.2	161.8	101.8	44.1

[a] Day before used as base value for calculations.

The chlorobutanol was placed directly in the rumen contents in crystalline form and was injected into the cecum and colon, dissolved in 10 c.c. of 95 per cent ethyl alcohol. As shown in table 8, the ascorbic acid content of blood and urine was followed daily throughout the trial. On the third and fourth days, blood samples were taken at 0, 1, 2, 4, and 24 hours to determine whether an immediate increase in blood ascorbic acid followed the administration of chlorobutanol. These results are shown in figure 3 and will be discussed later in this section.

As shown in table 8, blood ascorbic acid was not consistently affected during chlorobutanol treatment, but both whole blood and blood plasma ascorbic acid decreased significantly during the 3-day period following chlorobutanol administration. Urinary ascorbic acid was tripled by the fourth day of chlorobutanol treatment.

On the tenth and twelfth days (table 8) 16 g. of ascorbic acid were placed in the rumen and 4 g. in the colon and cecum; 2 g. of ascorbic acid were administered to each steer intravenously on the fourteenth day and 2 g. subcutaneously on the sixteenth day. These administrations were made approximately 30 minutes after the daily dose of chlorobutanol. As shown in figure 3, the increases in blood ascorbic acid following the administration of ascorbic acid were not markedly affected by simultaneous chlorobutanol treatment. The maximum blood levels of ascorbic acid were observed 30 minutes after ascorbic acid injection rather than at 15 minutes when chlorobutanol was not given; there was a tendency for the increased blood levels to be maintained longer.

TABLE 8

Administering chlorobutanol and ascorbic acid directly into various levels of the digestive tract

Day	Treatment[a]	Av. ascorbic acid of four steers[b]						
		Whole blood		Blood plasma		Urine		Recovery[d]
			Diff.[c]		Diff.[c]		Diff.[c]	
		(mg.%)	*(mg.%)*	*(mg.%)*	*(mg.%)*	*(mg.%)*	*(mg.%)*	(%)
1	None	0.490		0.348		89.9		
2	None	0.438		0.410		72.0		
3	Ch.[e]	0.435	−0.029	0.265	−0.114[f]	98.2	17.2	
4	Ch.	0.475	0.011	0.425	0.046	104.2	23.2	
5	Ch.	0.580	0.116[g]	0.438	0.059	153.8	72.8	
6	Ch.	0.482	0.018	0.322	−0.057	217.2	136.2	
7	Ch.	0.490	0.026	0.430	0.051	243.8	162.8[f]	
8	Ch.	0.558	0.094	0.390	−0.011	180.6	99.6	....
9	Ch.	0.445	−0.019	0.322	−0.057	198.4	117.4	
10	Ch.[h]	0.488	0.024	0.355	−0.024	284.1	203.1[g]	0.9
11	Ch.	0.503	0.039	0.372	−0.007	180.0	99.0	
12	Ch.[h]	0.510	0.046	0.400	0.021	454.1	373.1[g]	2.6
13	Ch.	0.508	0.044	0.508	0.129[f]	142.6	61.6	
14	Ch.[i]	0.504	0.040	0.438	0.059	706.4	625.4[g]	25.8
15	Ch.	0.505	0.041	0.442	0.063	255.8	174.8[f]	
16	Ch.[j]	0.415	−0.049	0.295	−0.084	521.2	440.2[g]	15.3
17	Ch.	0.418	−0.046	0.270	−0.109[f]	192.5	111.5	....
18	None	0.288	−0.176[g]	0.192	−0.187[g]	140.0	59.0	
19	None	0.368	−0.096	0.220	−0.159[g]	104.8	23.8	
20	None	0.565	0.101[f]	0.315	−0.064	206.1	125.1	
21	None	0.440	−0.024	0.382	−0.003	174.2	93.2	
22	None	0.588	0.124[f]	0.402	0.023	172.6	91.6	
23	None	0.525	0.061	0.410	0.031	107.7	26.7	

[a] Determinations made 24 hr. after each designated treatment.
[b] Represents two rumen, one colon, and one cecum fistula.
[c] Mean of days 1 and 2 used as base level.
[d] Mean of days 8 and 9 used as base level.
[e] Chlorobutanol.
[f] Significant mean difference—base levels compared to other means.
[g] Highly significant mean difference—base levels compared to other means.
[h] 4 g. ascorbic acid administered into cecum and colon, 16 g. into rumen.
[i] 2 g. ascorbic acid given intravenously.
[j] 2 g. ascorbic acid given subcutaneously.

The details of urinary elimination are shown in tables 8 and 9. Whereas in the previous trials digestive tract administration of ascorbic acid did not definitely increase urinary elimination, in these trials elimination, on the average, was 100 mg. higher for the first trial and 225 mg. higher for the second trial as compared with the day prior to treatment. The percentage of administered ascorbic acid recovered on the day of treatment was calculated on the basis that urine excretion on the eighth and ninth days (table 8) was an acceptable base for ascorbic acid elimination in urine from steers receiving chlorobutanol. The amount recovered (stated in per cent of total administered) is shown by steers and by trials in table 9. The recovery from digestive tract administration still was quite low, since rather large quantities of ascorbic acid were given. The recovery following intravenous administration was 25.8 per cent as compared to 12.2 per cent when these same steers were not receiving chlorobutanol; recovery following subcu-

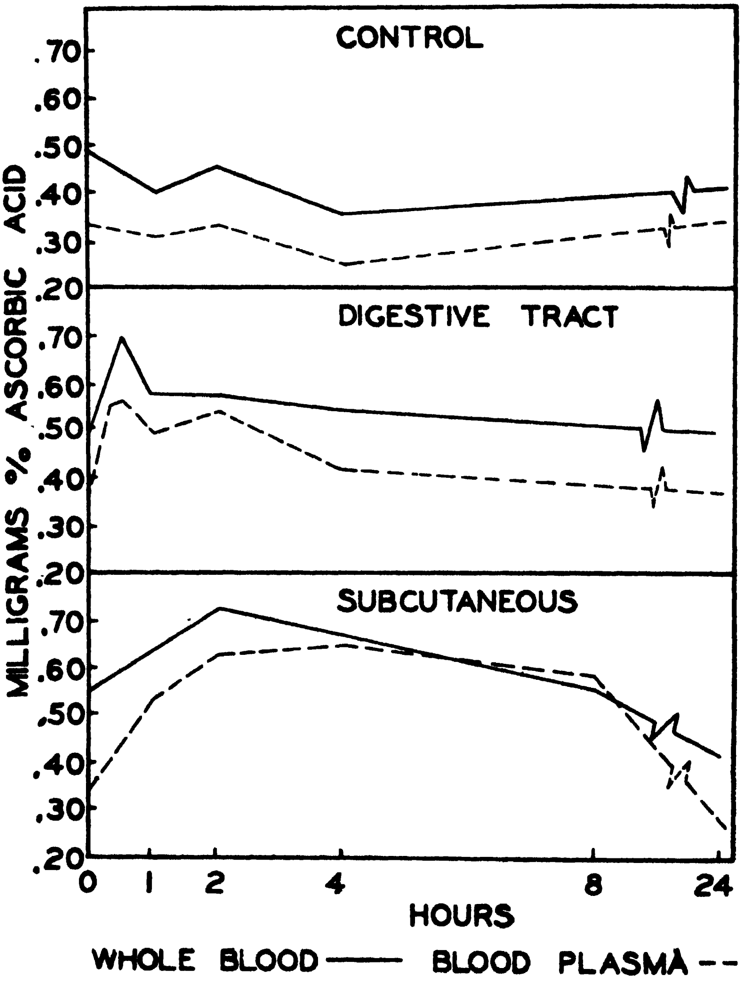

Fig. 3. The effect of chlorobutanol on blood ascorbic acid following ascorbic acid administration.

TABLE 9

Percentage of administered ascorbic acid recovered in the urine of steers receiving chlorobutanol

Steer	Percentage of ascorbic acid recovered after administration by way of:			
	Digestive tract[a]		Intravenous[b]	Subcutaneous[b]
	Trial 1	Trial 2		
	(%)	(%)	(%)	(%)
Colon	3.6	17.6	42.2	19.2
Cecum	0.4	2.6	20.6	4.9
Rumen—1	0.6	0.7	17.3	8.2
Rumen—2	0.8	0.9	23.2	29.0
Summary	0.9	2.6	25.8	15.3

[a] 4 g. of ascorbic acid placed in colon and cecum, 16 g. in rumens.
[b] 2 g. of ascorbic acid administered.

taneous administration also was 6 per cent higher than previously observed.

The data from this series of trials indicate that chlorobutanol increased urinary elimination of ascorbic acid approximately the same in all steers, even though administration was made in three different parts of the digestive tract, namely, rumen, colon, and cecum.

Subcutaneous administration of chlorobutanol. Base levels were established on the four steers used in the above studies. Three grams of chlorobutanol in 5 c.c. of 95 per cent ethyl alcohol then were injected subcutaneously into each steer daily for 5 days. Blood samples were taken daily and 24-hour urine specimens were collected. The results are shown in table 10. Whole blood and blood plasma ascorbic acid decreased considerably during the first 3 days of treatment. However, whole blood values

TABLE 10

Effect of administering chlorobutanol subcutaneously

Day	Treatment	Av. ascorbic acid of four steers					
		Whole blood		Blood plasma		Urine	
			Diff.[a]		Diff.[a]		Diff.[a]
		(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)
1	None	0.380		0.268		71.2	
2	None	0.445		0.330		94.4	
3	Ch.[b]	0.368	−0.044	0.250	−0.049	84.1	1.3
4	Ch.	0.332	−0.080[c]	0.160	−0.130[d]	87.2	4.4
5	Ch.	0.285	−0.127[d]	0.225	−0.074	99.5	16.7
6	Ch.	0.432	0.020	0.262	−0.037	182.8	100.0[d]
7	Ch.	0.500	0.088[c]	0.265	−0.034	165.0	82.2[d]

[a] Mean of days 1 and 2 used as base level.
[b] Determination 24 hr. after treatment with chlorobutanol (Ch.).
[c] Significant mean difference—base levels compared to other means.
[d] Highly significant mean difference—base levels compared to other means.

increased for the next 2 days, while blood plasma remained slightly lower than for the first 2 days, which were used as base levels for purposes of comparison. Urinary excretion was nearly doubled on the fourth day after treatment began and remained at a high level on the last day of the trial. Each steer responded equally well each of the 2 days. This trial was discontinued as soon as a definite increase in urinary excretion was noted, since it was not thought desirable to give chlorobutanol in 95 per cent ethyl alcohol solution subcutaneously for a prolonged period. No indications of sloughing in the regions of administration were apparent several weeks afterwards.

Blood ascorbic acid relationships. Blood cell ascorbic acid was calculated for all blood samples using a blood cell volume-hemoglobin ratio determined by the random selection of two blood samples from each of four steers. This ratio on 48 such samples averaged 2.145 ± 0.035. Hemoglobin determinations were made on all samples. Correlation and regression coefficients were determined between whole blood and blood plasma ascorbic acid values for the various bleeding intervals and treatments reported in this study. The original data for these studies are reported elsewhere (4). Results indicate that cellular elements of the blood are permeable to ascorbic acid. This was particularly apparent following intravenous administration. However, in all these trials (tables 1 and 2, figs. 1 and 2), whole blood ascorbic acid tended to increase more slowly and decrease less rapidly than blood plasma ascorbic acid. Calculated blood cell ascorbic acid varied considerably and seemed to have a cyclic rate of change. For example, in the case of intravenous administration, calculated blood cell ascorbic acid was quite high at 1, 2, and 4 minutes after treatment and then decreased in the next 12 minutes to considerably below base levels, slowly recovering thereafter. The magnitude of this phenomenon was not so pronounced in the other types of administration. The blood cell ascorbic acid content for these steers usually varied from approximately 0.7 to 1.0 mg. per cent during non-treatment periods but consistently showed values above and below during experimental periods. This indicates that the ascorbic acid balance between plasma and cells was upset and that some compensatory action was taking place.

Interval of bleeding on the hemoglobin content of blood. Hemoglobin values were observed to decrease rather uniformly during periods when blood samples were drawn frequently. Some of these data were summarized for the various treaments and various bleeding intervals. The results, as shown in table 11, reveal that hemoglobin was reduced significantly during the periods of rapid bleeding used in the control, digestive tract (rumen, colon, and cecum summary), small intestine, and intravenous studies. This drop in hemoglobin also has been observed recently in sheep by Thomson *et al.* (29). The *F*-ratio between bleeding intervals was highly significant. The bleeding intervals used during the subcutaneous studies did not show any apparent influence on the hemoglobin content of blood, the *F*-ratio being only 0.34. Since feeding and watering were done at the same relative time in all the studies, variation due to these factors should have been apparent in the subcutaneous trials. Furthermore, the steers drank

TABLE 11

Effect of bleeding interval on average hemoglobin in the blood of steers

Bleeding interval	No. of sample series	Av. Hb. (*gm.%*)	Diff.[a] (*gm.%*)
Control studies			
−24 hr.	8	13.94	
0		13.18	
15 min.		12.96	−0.75
30 min.		12.33	−1.38[b]
60 min.		12.78	−0.93[c]
90 min.		12.15	−1.56[b]
2 hr.		11.88	−1.83[b]
3 hr.		13.19	−0.52
4 hr.		14.42	0.71
6 hr.		13.42	−0.29
8 hr.		13.60	−0.11
24 hr.		14.01	
Digestive tract studies[d]			
−24 hr.	32	13.17	
0		13.60	
15 min.		13.58	0.41
30 min.		13.00	−0.17
60 min.		12.75	−0.42
90 min.		12.74	−0.43
2 hr.		12.30	−0.87[b]
3 hr.		12.76	−0.41
4 hr.		13.25	−0.08
6 hr.		12.61	−0.56[c]
24 hr.		12.74	
Small intestine studies			
−24 hr.	8	10.84	
0		11.03	
15 min.		9.67	−1.36[b]
30 min.		10.43	−0.60
60 min.		9.80	−1.23[b]
90 min.		8.99	−2.04[b]
2 hr.		9.28	−1.75[b]
3 hr.		9.86	−1.17[b]
4 hr.		9.70	−1.33[b]
6 hr.		9.93	−1.10[b]
24 hr.		11.20	
Subcutaneous studies			
−24 hr.	8	12.62	
0		13.51	
1 hr		13.02	−0.27
2 hr.		13.53	0.24
3 hr.		13.42	0.13
4 hr.		13.22	−0.07
6 hr.		13.46	0.17
8 hr.		12.91	−0.38
24 hr.		13.46	
Intravenous studies			
−24 hr.	8	13.34	
0		14.46	
1 min.		13.70	0.27
2 min.		13.94	0.51
4 min.		14.04	0.61
8 min.		13.76	0.33
16 min.		12.57	−0.86
32 min.		13.48	0.05
60 min.		13.15	−0.28
90 min.		13.03	−0.40
2 hr.		12.32	−1.11[c]
3 hr.		12.29	−1.15[c]
4 hr.		11.16	−2.27[b]
24 hr.		12.46	

[a] Mean of −24, 0, and 24 hr. used as base level.
[b] Highly significant mean difference.
[c] Significant mean difference.
[d] Four steer summary includes rumen 1 and 2, colon, and cecum.

very little water after the morning feeding; maximum water intake invariably took place after the evening feeding.

DISCUSSION

Absorption of ascorbic acid from the digestive tract. Riddell *et al.* (22) changed three dairy cows abruptly from a winter ration to one consisting of 65 kg. of green rye daily. They observed that ascorbic acid in blood plasma was more than doubled during the first 12 hours and that urine output increased five times within 60 hours. Later, Knight *et al.* (10) and Vavich *et al.* (32) fed or administered up to 150 g. of ascorbic acid to Holstein cows without observing any increases in blood plasma ascorbic acid in samples taken 1.5 hours after administering the vitamin. Vavich *et al.* (32) also fed dehydrated cereal grasses to one cow without influencing blood plasma ascorbic acid. Lundquist and Phillips (13) observed that ascorbic acid administered *per os* to calves would raise blood plasma levels for the first 10–12 days of life but was ineffective thereafter.

In this study a total of 76 trials were conducted on five steers. The administration of 16 g. of ascorbic acid into the ventral rumen sac significantly increased whole blood and blood plasma ascorbic acid within 15 minutes. Slight increases were noted for the first 4 hours, but these mean differences were not statistically significant. The administration of 4 g. of ascorbic acid into the cecum and colon also produced significant increases in blood ascorbic acid during the first 2 hours following treatment. The maximum values, however, generally were observed within the first 15 minutes. When the 32 trials (representing 12 rumen, 10 cecum, and 10 colon) were summarized, significant mean differences in whole blood and blood plasma ascorbic acid were observed at 15, 30, 60, and 90 minutes, and at 2 and 3 hours after administration of ascorbic acid. When 50 c.c. of distilled water were administered to these steers (8 trials) and the same bleeding routine followed, whole blood ascorbic acid was significantly decreased after 1, 1.5, and 4 hours, while blood plasma ascorbic acid was significantly lowered at 4 hours. Average urine elimination of ascorbic acid was significantly higher during the 24-hour period following treatment when 16 g. of ascorbic acid were placed in the rumen, but little or no increase was observed from placing 4 g. of the vitamin in the colon or cecum.

The administration of 4 g. of ascorbic acid into the small intestine also significantly increased ascorbic acid in whole blood and blood plasma for the first hour after treatment, with the maximum increase coming at 30 minutes.

The deposition of 16 g. of ascorbic acid in the reticulo-omasal-abomasal orifice also significantly increased ascorbic acid in whole blood and blood plasma and more than tripled urine elimination of ascorbic acid. Holding ascorbic acid against the rumen wall significantly increased whole blood and blood plasma ascorbic acid. Administration of 16 g. of ascorbic acid by stomach tube increased blood ascorbic acid in three out of six trials, but these increases were not significant. Likewise, urinary elimination was not noticeably increased by this treatment.

Though results in these studies are not in agreement with Knight *et al.* (10) and Vavich *et al.* (32), a direct comparison cannot be made. Maximum absorption of ascorbic acid in this study was observed within 15 minutes after treatment, whereas, Knight *et al.* (10) and Vavich *et al.* (32) apparently did not take blood samples prior to 1.5 hours after feeding or placing ascorbic acid in the rumen. The time lag between administration and bleeding could explain the failure to observe increased blood plasma ascorbic acid. However, these authors did observe slight increases in urine elimination of ascorbic acid following treatment.

Daily administration of 5 g. of chlorobutanol for 15 days into the rumen, colon, and cecum tripled the urinary output of ascorbic acid by the fifth day. This increase essentially was the same as that observed in dairy cattle by Moore and Cotter (17) and Moore (16), and in rats by Longenecker *et al.* (12) and Sutton *et al.* (28). The ascorbic acid content of whole blood and blood plasma was not increased. This may have been due to the short administration period, since Scheidenhelm *et al.* (26), Bortree *et al.* (2, 3), Lundquist and Phillips (14, 15), Moore and Cotter (17), and Moore (16) frequently observed animals that required chlorobutanol administration for more than 15 days before blood plasma ascorbic acid was raised. A pronounced drop in blood ascorbic acid occurred for several days immediately following the cessation of chlorobutanol treatment. This also was observed by the previous workers, but their observed decrease was not so rapid and recovery was much slower.

Ascorbic acid, when administered subcutaneously, intravenously, or into the respective fistulae of these chlorobutanol-conditioned steers, resulted in a marked increase in the per cent of administered ascorbic acid recovered as compared to recovery from the same steers when not receiving chlorobutanol. Ascorbic acid in the urine increased, on the average, 100 to 200 mg. when ascorbic acid was placed in the digestive tract following chlorobutanol treatment as compared to much smaller increases when the steers were not receiving chlorobutanol. Intravenous recovery of ascorbic acid was increased from 12.2 to 25.8 per cent and subcutaneous recovery from 9.3 to 15.3 per cent by chlorobutanol treatment. The quantities of ascorbic acid found in venous blood did not seem to be appreciably greater. However, peak values generally were observed at 30 instead of 15 minutes. Knight *et al.* (10) gave 24 g. of ascorbic acid to cows subcutaneously and intravenously for 3 successive days. They recovered about 25 per cent of the ascorbic acid in the case of intravenous administration and slightly less in subcutaneous administration, according to our calculations from their original data. In the present study only 2 g. of ascorbic acid were used and for only 1 day. The magnitude of recovery was less in this case except when administration was made to chlorobutanol-conditioned animals. The low rate of recovery of ascorbic acid in urine, even when the vitamin was placed directly in the blood stream at the rate of 24 g. per day for 3 successive days (10) suggests that the bovine does not readily eliminate ascorbic acid in the urine. Several possibilities exist: the vitamin could be eliminated in feces, it could be absorbed by other body tissues, or it could be used as a metabolite. Feces studies (4) do not suggest that any appreciable fecal elimination takes place. Ascorbic acid also could be oxidized to the diketogulonic acid or other non-reversible oxidative forms and thus not be detected by the methods of analysis used. The extensive studies of Borsook *et al.* (1) show quite clearly that ascorbic acid is quite stable in blood and tissues and that dehydroascorbic acid is reduced rapidly in minced tissues. This reducing substance further was shown to be glutathione. This does not rule out the possibility that, when excessive amounts of ascorbic acid suddenly are placed in the blood stream, the non-reversible oxidative forms would not be produced. It seems more than probable, however, that the animal body can use excess quantities of ascorbic acid as a metabolite. It would be difficult to determine how much ascorbic acid was absorbed from digestive tract administration. The mechanism by which chlorobutanol causes increased ascorbic acid elimination in urine and raises blood levels is unknown. Whether the fact that recovery of administered ascorbic acid is markedly increased in steers receiving chlorobutanol adds anything to the problem is difficult to say. If the theory is accepted that body synthesis is increased, then increased urinary elimination of administered ascorbic acid could be explained by the fact that the body has less use for the extra ascorbic acid, which then would be eliminated. On the other hand, chlorobutanol may merely decrease the ability of the organism to utilize the vitamin and in this manner cause increased ascorbic acid elimination. Feng (6) reported that feeding chlorobutanol to rats increased the ascorbic acid content of adrenals, kidneys, intestine and liver over the controls. This would suggest that ascorbic acid is not driven out of the tissues. The observations by Moore and Cotter (17) that pregnant heifers on a low carotene ration excreted more ascorbic acid in the urine than pregnant heifers fed normally and that such heifers did not show any further increase when fed chlorobutanol also are of speculative interest.

When the digestive tract is by-passed by administering chlorobutanol subcutaneously, urinary elimination of ascorbic acid is increased as rapidly as when the drug was placed in the digestive tract. No apparent changes occurred in blood ascorbic acid during the 5-day treatment period.

The rate of removal of ascorbic acid from the blood stream. Bleeding steers 10 times in a single 8-hour period significantly reduced the ascorbic acid content of whole blood and blood plasma ascorbic acid. Increases in blood ascorbic acid resulting from administering the vitamin directly into the digestive tract were frequently noted at 15 and 30 minutes after treatment but not at 60 minutes or at any time thereafter. Subcuaneous administration of 2 g. of ascorbic acid was followed by maximum blood levels in 3 to 6 hours, with return to base levels at 24 hours. When 2 g. of ascorbic acid were given intravenously, approximately one-half was removed from venous blood in 4 minutes and half of the remainder in the next 12 minutes, and this again was reduced by half at the end of 32 minutes. While somewhat higher levels of blood ascorbic acid were observed 4 hours after administration as compared to the base period, these values had returned to base levels after 24 hours.

Blood cell and blood plasma ascorbic acid relationships. Borsook *et al.* (1) studied permeability of red blood cells by adding ascorbic acid to oxalated whole blood and incubating for 1 hour at 37.5° C. They concluded that the red corpuscles of beef cattle, cats, dogs, humans, pigs, rats and sheep are nearly, if not absolutely, impermeable to added ascorbic acid. There were some slight indications, however, that the red corpuscles of pigs and sheep had some permeability.

Results in this study indicate that the cellular elements of the blood do absorb some ascorbic acid. This was particularly apparent during the first 4 minutes following intravenous administration. It also was noted during subcutaneous and digestive tract administration. However, in all these trials, whole blood ascorbic acid tended to increase more slowly and decrease less rapidly than blood plasma ascorbic acid. Calculated blood cell ascorbic acid varied considerably and seemed to have a cyclic rate of change. For example, in the case of intravenous administration, calculated blood cell ascorbic acid was quite high at 1, 2, and 4 minutes after treatment and then decreased in the next 12 minutes to considerably below base levels, and then recovered slowly thereafter.

SUMMARY

1. The ascorbic acid content of whole blood and blood plasma was significantly increased within 15 minutes after placing ascorbic acid into the rumen, reticulo-omasal-abomasal orifice, small intestine, cecum, and colon. These increases persisted for only 2–3 hours.

2. Holding ascorbic acid solution against the rumen wall significantly increased the ascorbic acid content of whole blood and blood plasma.

3. Very little ascorbic acid was recovered in the urine following digestive tract administration, and only 9.2 and 12.3 per cent, respectively, were recovered following subcutaneous and intravenous administration. The recovery of ascorbic acid was greatly increased when these types of administration were made to steers receiving chlorobutanol.

4. The administration of chlorobutanol into the rumen, colon, and cecum at the rate of 5 g. daily for 15 days or subcutaneous administration at the rate of 3 g. daily for 5 days significantly increased urinary elimination

of ascorbic acid the fourth day after the start of treatment. Blood ascorbic acid was not increased by either method of chlorobutanol administration.

5. Calculations made to determine the ascorbic acid content of blood cells indicated that the vitamin passed freely between blood cells and blood plasma, although in the case of subcutaneous or intravenous administration blood plasma values were at times considerably higher than for whole blood.

6. The hemoglobin content of blood was significantly decreased by bleeding cattle at frequent intervals.

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THE PHARMACODYNAMICS OF ASCORBIC ACID IN CATTLE. II. *IN VIVO* AND *IN VITRO* LOSS[1, 2]

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Investigations by Whitnah and Riddell (13), Knight *et al.* (6), and Vavich *et al.* (11) have shown that ascorbic acid rapidly is lost in the rumen contents of cattle both *in vivo* and *in vitro.* Kendall and Chinn (4) were unable to show *in vitro* decomposition of ascorbic acid in filtered rumen juice obtained from freshly slaughtered cows. Knight *et al.* (6) found that ascorbic acid rapidly disappeared from the rumen contents of a cow (*in vivo*) after she was fed as much as 150 g. of crystalline ascorbic acid. Studies of rumen juice incubated at 39–42° C. revealed that loss of added ascorbic acid was rapid but not as rapid as *in vivo.* This was attributed to lack of continuous mixing *in vitro* compared to continuous mixing *in vivo.*

Vavich *et al.* (11) found that ascorbic acid was destroyed quite slowly in sterilized rumen contents. When dehydrated cereal grasses were fed as a source of ascorbic acid, the vitamin could be demonstrated in the rumen more than 8 hours after feeding, but the highest concentration occurred 2 to 4 hours after feeding. Orally administered crystalline ascorbic acid could be recovered for approximately 2 hours. These authors also removed all rumen contents, flushed the rumen with warm water, and again emptied it. The cow then was fed a low ascorbic acid ration. *In vivo* and *in vitro* studies of this material showed little loss of ascorbic acid, but when 2 gallons of the fermenting rumen contents were returned to the rumen, definite loss of added ascorbic acid occurred. Young and James (14) and Young and Rettger (15) have reported that human fecal cultures and isolated cultures of *Escherichia coli* and *Aerobacter aerogenes* decomposed ascorbic acid under both aerobic and anaerobic conditions. Organisms of the *Alcaligenes* and *Proteus* groups did not attack ascorbic acid and protected it from atmospheric oxidations but not from bacteria decomposing ascorbic acid. Glucose and lactose "spared" ascorbic acid from oxidation by fecal bacteria. Ascorbic acid-decomposing bacteria which produced gas from ordinary carbohydrates also produced gas when attacking ascorbic acid. Stepp and Schroder (10) also have reported on a strain of *Escherichia coli* that decomposed ascorbic acid.

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The pH in various parts of the digestive tract is quite variable. Dukes (1) reports that most rumen studies of cattle show a range of pH 6.0 to 7.8. Kick *et al.* (5), working with steers, reported that their determinations varied with rations fed, ranging from pH 5.5 to 7.7, with the most alkaline reaction being on material from animals fed alfalfa alone. Monroe and Perkins (7) reported an average pH value between 6.83 and 7.01 for animals fed such roughages as corn or A.I.V. silage and alfalfa hay. They found that when samples were taken at 2-hour intervals throughout the day under dry lot conditions, the pH was most alkaline just before the morning feeding, with the largest variations occurring just after feeding. Smith (9) made *in vivo* studies on two cows and found that rumen pH was 6.30 on an alfalfa ration and 6.07 on alfalfa and beet pulp. The pH generally was more alkaline shortly before and after feeding and varied from 6.27 in the front to 6.00 in the rear of the rumen.

The available literature on rumen flora and fauna has been excellently reviewed by Hastings (3). In general, numbers of bacteria have been difficult to estimate, since many do not grow on the usual laboratory media. Protozoa concentrations have been reported variously from 500 million to 1 billion per ml. These organisms have been estimated to comprise as much as 10 per cent of the total dry matter in the rumen.

EXPERIMENTAL

Data on the rate of removal or loss of ascorbic acid from the contents of various portions of the digestive tract were gathered simultaneously with the data on absorption and elimination of ascorbic acid, which were reported in the previous paper (2). Rumen juice, small intestinal juice, cecal and colon contents, and the feces were studied. Ascorbic acid was determined by the method of Roe and Keuther (8) with such modifications as were necessary to give satisfactory dilutions. All colorimetric determinations were made with the Evelyn photoelectric colorimeter. All pH determinations were made with a glass electrode pH meter.

Samples of rumen juice were taken from the ventral rumen sac. Cecal and colon contents were allowed to ooze from the fistulae into 50 c.c. flasks. These samples were taken immediately to the laboratory for pH and ascorbic acid determinations.

The ascorbic acid content and pH of the digestive tract contents before and following the administration of ascorbic acid are shown in table 1. The pH range of 12 determinations on the rumen contents was 5.72 to 6.56, and 6.92 to 8.12 in the colon in untreated animals. The administration of 16 g. of ascorbic acid in the ventral rumen sac did not lower the pH of the rumen juice of the two steers. When the ascorbic acid level of the colon contents was above 300 mg. per cent, pH appeared to be lowered in several instances and ranged between 5.88 and 7.76. The contents from the cecal fistula did

TABLE 1

Ascorbic acid recovery in vivo

Administration		Av. and no. of cases	Hr. from treatment											
			−24	0	0.25	0.5	1	1.5	2	3	4	6	8	24
(site)	*(g.)*													
Rumen—2	0	No. A.A.mg.% pH		2 0.12 6.10	2 0.10 6.23	2 0.03 6.10	2 0.06 6.44	2 0.04 6.49	2 0.06 6.32					2 0.22 6.10
Colon	0	No. A.A.mg.% pH		2 0.28 7.42	2 0.78 7.66	2 0.25 7.28	2 0.55 7.41	2 0.40 7.31	2 0.13 7.52					2 0.40 7.29
Rumen—1	16	No. A.A.mg.% pH	2 1.28 6.45	4 1.21 6.80	3 95.0 6.72	4 30.0 6.88	5 19.0 6.96	3 1.62 6.51	4 1.09 6.87	4 1.26 6.68	4 1.12 6.55	2 0.14 7.18		3 0.91 6.34
Rumen—2	16	No. A.A.mg.% pH	2 1.20 6.46	4 1.02 6.72	3 194.9 6.56	4 135.0 6.50	5 42.2 6.75	3 55.0 6.43	4 15.2 6.82	4 1.24 6.51	4 0.90 6.57	2 0.16 6.35		3 0.82 6.44
Rumens—1–2		A.A.—range	0.49–1.90	0.27–1.80	63.7–407.0	21.0–317.0	0.39–133.0	0.77–95.0	0.24–59.0	0.23–2.50	0.20–1.90	0.11–0.21		0.06–1.90
		pH—range	6.28–6.64	6.34–7.38	6.34–6.84	6.24–7.38	6.24–7.46	5.74–6.94	6.24–7.20	6.08–7.18	6.08–7.16	6.18–7.24		6.12–6.64
Colon	4	No. A.A.mg.% Range	4 2.10 0.10–5.9	5 2.39 0.28–7.2	5 268.7 32.4–693.0	5 37.1 4.6–283.0	7 38.3 3.7–96.4	5 21.7 2.4–54.9	5 59.1 2.5–166.2	5 3.80 1.2–6.1	6 3.88 0.81–6.0	4 1.78 0.99–3.2	4 0.62 0.38–0.93	5 1.70 0.12–5.90
		No. pH Range	2 7.20 7.64–6.76	4 7.31 6.98–7.52	4 6.82 5.88–7.54	3 7.35 7.04–7.64	5 7.15 6.82–7.48	5 7.12 6.64–7.60	3 7.33 7.08–7.46	2 6.89 6.84–6.94	5 6.91 6.72–7.32	1 7.02	1 7.42	3 7.0 6.64–7.76
Cecum	4	No. A.A.mg.% Range	1 0.21	3 1.15 0.60–2.11	4 327.7 29.4–528.0	2 264.8 28.4–501.2	3 301.5 111.0–573.8	2 38.8 18.1–59.5	3 29.4 3.4–62.5	2 158.5 120.3–196.8	4 71.3 9.4–112.8	1 8.4	3 3.8 0.80–1.90	3 9.3 0.21–27.4
		No. pH Range		1 6.70	2 6.72 6.46–6.97		2 6.83 6.74–6.92		2 7.42 7.02–7.82	1 7.12	2 7.73 7.02–8.45			1 7.26

not ooze from the opening at a constant rate, and fewer determinations were made from this area. The pH range following ascorbic acid treatment was 6.46 to 8.45. Considerable quantities of ascorbic acid were recovered in the rumen juice during the first 2 hours following administration of 16 g. and in the colon contents during the first 4 hours after injecting 4 g. of ascorbic acid. In the cecum appreciable amounts of ascorbic acid were present 24 hours after administration.

The effects of subcutaneous and intravenous administration of ascorbic acid upon the amount of ascorbic acid in the digestive tract contents are shown in table 2. Ascorbic acid treatment by these routes had no apparent effect on either ascorbic acid content or pH. The administration of chlorobutanol into the rumen, colon, and cecum at the rate of 5 g. daily for 9 days had no effect on pH or ascorbic acid content of the ingesta.

The ascorbic acid content of freshly voided feces was determined prior to treatment and following administration of ascorbic acid and chlorobutanol. These findings are summarized in table 3. The administration of ascorbic acid into the rumen, cecum, and colon appeared to increase fecal elimination of ascorbic acid. Large quantities of ascorbic acid appeared in the feces 8 hours after administration into the cecum. This was not so apparent in the case of the rumen- and colon-fistulated steers, although fecal ascorbic acid appeared to be higher up to 24 hours. Possibly the sampling time of 8 hours after treatment was too long in the case of the colon and too short an interval after rumen administration. However, oxidation and dilution of ascorbic acid placed in the rumen would be expected to decrease greatly the possibility of such treatment increasing fecal ascorbic acid. The subcutaneous and intravenous administration of ascorbic acid had little if any effect on the feces. Chlorobutanol, when placed in the various fistulae, had no apparent effect on the ascorbic acid content of the feces.

A few determinations were made on small intestinal contents. These results are shown in table 4. Results of four determinations made before treatment with 4 g. of ascorbic acid showed ascorbic acid to be no higher than was found in rumen juice. The pH, however, was quite alkaline, ranging from 7.22 to 8.16 on 12 samples and averaging 7.63. Considerable ascorbic acid was found in the contents 1.5 hours after administration of the vitamin. Samples were not taken sooner because of the necessity of keeping the fistula-lumen plugged for this period of time after administration to prevent loss of the vitamin. Administration also was made posteriorly into the gut, so that most of the ascorbic acid probably passed too far back to leak out of the fistula when ingesta samples were taken.

Rate of loss of ascorbic acid added in vivo and incubated in vitro at 37° C. In the previous section it was shown that samples of digestive tract contents removed at regular intervals after administration of ascorbic acid contained appreciable quantities of the vitamin for at least 2 hours. To determine the

TABLE 2

The effect of administering ascorbic acid, subcutaneously and intravenously, and chlorobutanol into the digestive tract

Treatment		Av. and range	Steer			
			Colon	Cecum	Rumen—1	Rumen—2
(*type*)	(*g.*)					
Ascorbic acid subcutaneously	2	A.A.[a]mg.%	0.69 (17)[b]			0.24 (16)
		Range	0–1.68			0.04–0.75
		pH	7.59 (14)			6.60 (16)
		Range	7.02–8.00			6.00–7.26
Ascorbic acid intravenously	2	A.A.mg.%	0.57 (8)			0.29 (8)
		Range	0.23–0.95			0.04–1.35
		pH	7.26 (5)			6.39 (8)
		Range	6.86–7.52			5.88–6.52
Chlorobutanol in digestive tract	5	A.A.mg.%	0.64 (10)	0.62 (4)	0.19 (11)	0.31 (11)
		Range	0.30–0.88	0.15–1.12	0–0.45	0–1.50
		pH	7.15 (8)	6.85 (2)	6.94 (9)	6.83 (9)
		Range	6.86–7.52	6.68–7.02	6.70–7.08	6.48–7.12

[a] Ascorbic acid.

[b] Figures in parentheses indicate number of determinations.

TABLE 3

The effect of administering ascorbic acid and chlorobutanol on the ascorbic acid content of the feces

Treatment		Time interval	Av. and range of ascorbic acid	Steer			Summary[a]
				Colon	Cecum	Rumen—1–2	
(type)	*(g.)*	*(hr.)*		*(mg.%)*	*(mg.%)*	*(mg.%)*	*(mg.%)*
Ascorbic acid in digestive tract	4 and 16[b]	0	Av.	1.12 (3)[c]	2.40 (3)	2.16 (6)	
			Range	0.18–2.00	0.59– 3.80	0.70– 3.90	
		8	Av.	2.17 (3)	43.7 (3)	4.55 (5)	
			Range	1.32–2.80	25.0 –70.8	2.10–10.60	
		24	Av.	2.38 (2)	2.04 (2)	1.58 (4)	
			Range	0.47–4.30	0.68– 3.40	0.43– 2.80	
Ascorbic acid subcutaneously	2	0	Av.				0.854 (8)
			Range				0.60–1.08
		8	Av.				0.652 (8)
			Range				0–1.60
		24	Av.				0.365 (4)
			Range				0.23–0.51
Ascorbic acid intravenously	2	0	Av.				1.20 (4)
			Range				0.64–1.92
		4	Av.				1.78 (4)
			Range				1.12–2.38
Chlorobutanol in digestive tract	5	Daily	Av.	0.80 (7)	0.69 (8)	0.68 (15)	0.712 (30)
			Range	0.47–1.30	0.24–0.98	0–1.80	0–1.80

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[a] Same number of samples from each steer.

[b] 4 g. in colon and cecum; 16 g. in rumen.

[c] Figures in parentheses indicate number of determinations.

TABLE 4

In vivo recovery of ascorbic acid from a jejunal fistula and the pH value of the contents

Av. and range	Time interval—hr. after treatment				
	0	1.5	2	3	24
A.A.mg.%	0.28 (4)[a]	28.7 (4)	0.70 (3)	0.78 (1)	0.54 (3)
Range	0.19–0.34	9.0 –43.1	0.23–0.98		0.28–0.73
pH	7.65 (3)	7.65 (3)	7.62 (3)	7.16 (1)	7.84 (2)
Range	7.54–7.86	7.22–8.02	7.32–7.92		7.52–8.16

[a] Figures in parentheses indicate number of determinations.

rate of loss of ascorbic acid in ingesta outside the body, trials were conducted on contents from the small intestine, colon, and rumen following administration of ascorbic acid into these regions.

Samples of the rumen, small intestinal, and colon contents were collected in flasks, tightly stoppered except during sampling, and pH and ascorbic acid determinations were made immediately. The samples then were placed in a water bath at 37° C. and ascorbic acid determinations made after 2, 4, 6, 11, and 23 hours of incubation. The colon and rumen contents were obtained from the fistulae 15 minutes after the administration of 4 and 16 g. of ascorbic acid into the colon and rumen, respectively, and the small intestinal contents were secured 1.5 hours following the injection of 4 g. of the vitamin.

The results are shown in figures 1 and 2. Two widely different concentrations of ascorbic acid were secured for the incubation studies on small intestinal contents. One series of four trials showed an average of 42.6 mg. per cent ascorbic acid prior to incubation; after 6 hours of incubation approximately one-half of the ascorbic acid remained. Half of the remainder was lost in the next 5 hours. The other series of four trials had only 2.8 mg. per cent ascorbic acid prior to incubation and lost, on the average, approximately one-half of the ascorbic acid in 4 hours.

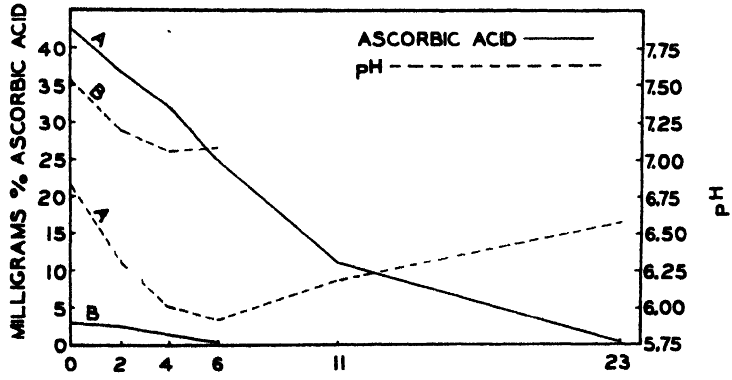

FIG. 1. *In vitro* loss of ascorbic acid and pH change in contents of the small intestine incubated at 37° C.—ascorbic acid added *in vivo*.

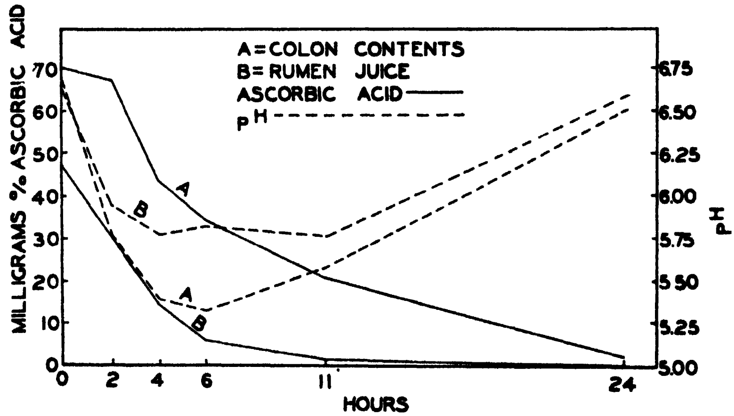

FIG. 2. *In vitro* loss of ascorbic acid and pH change in contents of the colon and rumen incubated at 37° C.—ascorbic acid added *in vivo*.

The average rate of loss of ascorbic acid from colon contents was approximately the same as for small intestinal contents. More ascorbic acid was left after 11 hours of incubation, but considerably more was present prior to incubation. The rate of loss of ascorbic acid from rumen juice, as shown in figure 2, was essentially twice as rapid as that in small intestinal and colon contents. The pH in all cases dropped for the first 4 to 6 hours and then gradually increased throughout the remainder of the incubation period.

Results from the previous section indicated that ascorbic acid gradually was lost from digestive tract contents over a period of 11 to 23 hours, the length of time depending on the amount originally in the incubated sample. Since it was not possible to get samples of uniform concentration from the *in vivo* method of mixing, ascorbic acid was added to rumen juice and colon contents in the laboratory. Samples of the rumen, small intestinal, and

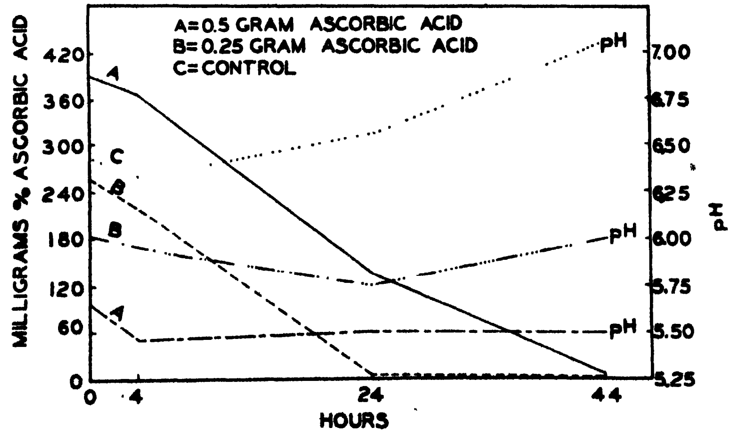

FIG. 3. pH change and loss of ascorbic acid added *in vitro* to rumen juice and incubated at 37° C.

colon contents were collected and divided into three portions of 100 c.c. each. One portion was used as a control, 0.25 g. of ascorbic acid was added to another, and 0.5 g. of ascorbic acid to the third. The ascorbic acid was mixed into the contents by shaking in tightly stoppered bottles, and a sample for pH and ascorbic acid determinations was removed and the remainder placed in a water bath at 37° C. The results of six trials on rumen juice and four trials on colon contents are shown in figures 3 and 4. The amount of ascorbic acid determined after addition of ascorbic acid and at the start of incubation was not any higher than quantities that occasionally were recovered from the digestive tract after vitamin administration. The average rate of loss was surprisingly constant for the first 24 hours in the rumen juice for both the high and low ascorbic acid samples, approximately 250 mg. of ascorbic acid being lost in each case. The average rate of loss of ascorbic acid in colon contents was slightly higher than the loss in rumen juice during the first 22 hours in the sample containing 0.5 g. of ascorbic acid. However, the colon samples which contained 0.25 g. of ascorbic acid showed an average loss approximately one-third as great; after 44 hours of incubation the amount of ascorbic acid remaining was, on the average, nearly the same in the two samples. The pH was lowered considerably by the addition of ascorbic acid. This decrease was greater than in the case of *in vivo* samples taken after ascorbic acid administration, even though the amount of ascorbic acid in these *in vivo* samples was in some cases higher than the concentration in the *in vitro* samples.

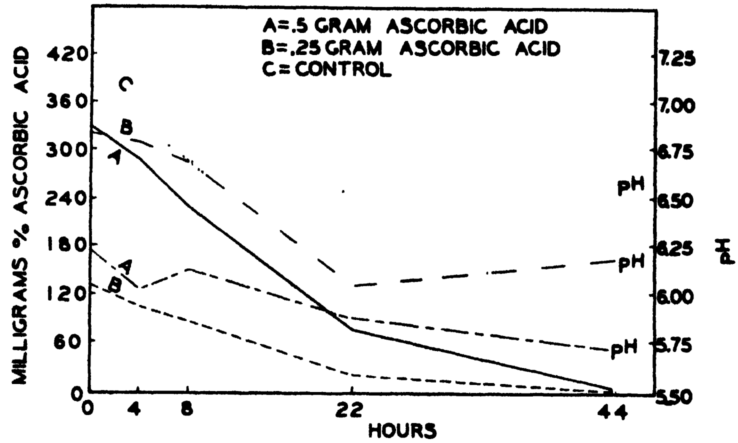

FIG. 4. pH change and loss of ascorbic acid added *in vitro* to colon contents and incubated at 37° C.

The effect of adding starch, autoclaving, and filtering rumen juice on loss of added ascorbic acid. Rumen juice samples for this study were taken from a Jersey cow with a rumen fistula during the last week of May, 1946. This cow was maintained on bluegrass pasture and received no grain. Approximately 1 l. of rumen juice was removed for each trial. Samples were prepared for incubation in screw capped jars and ascorbic acid was added at the rate of 4 mg. per 100 c.c. of material, except in the case of the bacteria-free filtrate, in which ascorbic acid was added at the rate of 8 mg. per 100 c.c. of material.

Corn starch was added at the rate of 50 g. per l. of ingesta. Samples were autoclaved at 15 lb. pressure for 30 minutes. Approximately 50 c.c. of bacteria-free filtrate were prepared by filtering through cotton and then through a Seitz filter. Fifty milligrams of chlorobutanol and thiourea were added to some samples singly and together. These two materials had no effect on the rate of loss of added ascorbic acid. The factors studied, as shown in figure 5, were starch added *vs.* no starch, ascorbic acid alone *vs.* ascorbic acid and starch, autoclaving plus ascorbic acid, and bacteria-free filtrate plus ascorbic acid. A control series also was made on autoclaved and bacteria-free filtrate

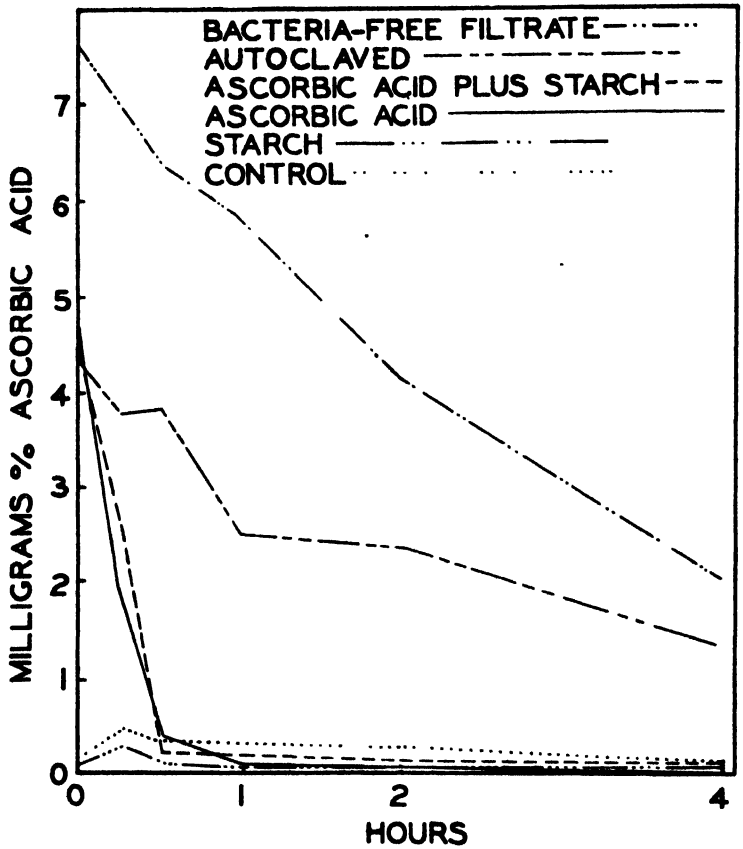

FIG. 5. The effects of starch, autoclaving, and removal of bacteria on rate of loss of ascorbic acid *in vitro* in rumen juice incubated at 37° C.

without added ascorbic acid. In the former, ascorbic acid was approximately twice as high as in untreated samples. The bacteria-free filtrate contained no more ascorbic acid than untreated rumen juice.

The results reveal that the added ascorbic acid was lost in untreated and starch-treated rumen juice in 30 minutes, with the latter showing less loss at 15 minutes than the former. The rate of loss in autoclaved and filtered material was considerably slower. Although precautions were taken to prevent bacterial contamination, some undoubtedly occurred in each case. However, the rate of loss in these samples would indicate that factors other than rumen flora alone may be involved in the loss of ascorbic acid in rumen juice.

DISCUSSION

In vivo. Samples of ingesta from the various fistulae taken during the control series showed pH values of rumen juice ranging from 5.72 to 6.56; the range of all pH determinations during all trials was 5.72 to 7.46. This range of values is essentially that reported by Dukes (1), Kick *et al.* (5), Monroe and Perkins (7), and Smith (9). The pH values of colon and cecum contents generally were between 7.00 and 8.00 except when large quantities of ascorbic acid were present. The pH of jejunal contents also was alkaline, showing a range of 7.22 to 8.16.

Ascorbic acid was recovered from samples of rumen juice for 2 hours following administration of 16 g. of the vitamin. This is essentially in agreement with Vavich *et al.* (11), although these authors administered as much as 150 g. of ascorbic acid at one time. Ascorbic acid was recovered from the colon 4 hours after treatment and for as long as 24 hours in cecal contents.

The administration of ascorbic acid subcutaneously and intravenously or of chlorobutanol into various levels of the digestive tract had no noticeable effect on the ascorbic acid content of rumen or colon contents.

There was evidence that ascorbic acid was eliminated in the feces in increased amounts following administration of ascorbic acid into the rumen, colon, and cecum, with the latter showing considerably more elimination at 8 hours after treatment.

Fecal elimination of ascorbic acid did not increase following the addition of chlorobutanol to the rumen, colon, and cecum. Neither was there any evidence that the subcutaneous or intravenous administration of ascorbic acid resulted in any increased fecal elimination. During these trials the range was from 0 to 2.38 mg. per cent ascorbic acid. The amount of ascorbic acid in the feces of the steers was several times less than that reported for dairy cows by Wallis (12). However, the age, type of animal, difference in ration, environment, and the presence of fistulae in the steers prevent any detailed comparisons between the two experiments.

In vitro. Whitnah and Riddell (13), Knight *et al.* (6), and Vavich *et al.* (11) observed rapid loss of ascorbic acid from rumen contents. Young and

James (14) and Young and Rettger (15) observed that certain organisms of the intestinal flora utilized or aided in the loss of ascorbic acid.

Incubation of digestive tract contents containing ascorbic acid added *in vivo* revealed that most of the vitamin was lost from rumen juice after 11 hours of incubation. Appreciable quantities remained in colon contents after 24 hours of incubation. Loss from small intestinal contents occurred at approximately the same rate as was observed in the case of colon contents.

The addition of ascorbic acid to rumen juice at the rate of 0.25 and 0.5 g. per 100 c.c. and incubation at 37° C., indicated approximately 250 mg. of ascorbic acid were lost from each sample during the first 24 hours and, in the case of the higher concentration, the remainder was lost during the next 20 hours. When 0.5 g. of ascorbic acid was added to 100 c.c. of colon contents and incubated at 37° C., the rate of loss approximated that observed for rumen juice. However, the samples containing only half as much ascorbic acid lost the vitamin only one-third as fast. The rate of loss of ascorbic acid added to rumen juice was considerably less than was observed by Vavich *et al.* (11). However, these authors incubated their samples at 39.5° C. and determined only reduced ascorbic acid.

Though the concentration of ascorbic acid was approximately twice as high in feces and in the colon and cecal contents as compared to rumen juice or juice from the jejunum, this does not necessarily mean that any lower bowel synthesis of ascorbic acid has taken place. The increase in dry matter in the lower bowel easily could account for the differences noted. Furthermore, to the authors' knowledge, no information is available on how much ascorbic acid might be found in the living microflora of the digestive tract. According to Hastings (3), the amount of microflora on a dry-matter basis is approximately the same for rumen and fecal material. Thus differences in ascorbic acid could be due to the concentration of microflora. The other possibility is the liberation of the vitamin from undigested portions of grain and hay as they proceed along the digestive tract. Getting more of this solid material in the sample easily could account for the differences observed.

Further studies on rumen juice revealed that ascorbic acid was lost quite slowly from a bacteria-free filtrate and autoclaved samples as compared to samples not treated in this manner. This essentially is in agreement with Vavich *et al.* (11).

The addition of corn starch to rumen juice of a cow on pasture and receiving no grain showed a slight "sparing" effect on ascorbic acid. Adding thiourea or chlorobutanol or both to rumen juice had no effect on the rate of loss of ascorbic acid. These studies indicate that both microbiological and chemical factors are involved in the loss of ascorbic acid from rumen juice. This loss cannot be attributed solely to bacteria or enzymes.

SUMMARY

1. The ascorbic acid content of rumen juice, intestinal juice, colon and cecal contents, and feces was determined during ascorbic acid absorption studies with steers.

2. Ascorbic acid was recovered from rumen juice for 2 hours following rumen administration of 16 g. of ascorbic acid. The vitamin was recovered for 4 and 24 hours, respectively, from colon and cecal contents after placing 4 g. of the vitamin directly into these regions.

3. The average ascorbic acid content of feces, cecal, and colon contents was approximately twice as high as jejunal or rumen juice during periods when ascorbic acid was not administered.

4. Placing chlorobutanol in the digestive tract or giving ascorbic acid subcutaneously or intravenously had no apparent effect on the level of ascorbic acid in digestive tract materials.

5. Some fecal elimination of ascorbic acid was evident following administration of ascorbic acid into the digestive tract.

6. *In vivo* and *in vitro* studies on rumen and jejunal juice and on colon contents indicated ascorbic acid was quite labile in these media, even though considerable quantities were recovered *in vivo* several hours after treatment. *In vitro* samples incubated at 37° C. lost ascorbic acid less rapidly.

7. Some ascorbic acid administered into the rumen, colon, and cecum was eliminated in the feces, with the latter showing the greatest elimination.

8. Autoclaving or preparing bacteria-free filtrates of rumen juice greatly retarded loss of ascorbic acid as compared to untreated rumen juice.

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OBSERVATIONS ON VITAMIN A DEFICIENCY IN YOUNG DAIRY BULLS[1]

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Vitamin A deficiency results in the cessation of spermatogenesis and atrophy of the testes in laboratory animals (11, 14), and recent studies (2, 3, 4, 5, 9, 10, 12, 13) have shown vitamin A deficiency to have a similar effect on the testes of the bull. It also has been reported (2, 3, 5, 6, 9, 10) that damage to germinal tissue of the testis due to vitamin A deficiency is reparable. The development of pituitary cysts has been attributed to vitamin A deficiency (5, 10, 12); this condition appears to be irreparable by vitamin A therapy. The common clinical symptoms of vitamin A deficiency are apt to occur prior to any recognizable reduction of fertility of bulls (5, 6, 9), especially when the deficiency develops after puberty.

The purpose of this study was to determine the effect of suboptimum intake of vitamin A upon reproduction in the male bovine and to determine whether or not prepubertal vitamin A deficiency results in permanent impairment of the reproductive processes which cannot be overcome by vitamin A therapy.

PROCEDURE

Two trials were conducted between August, 1944, and December, 1946. The first trial consisted of two groups of five bulls each, and the second trial of three groups of four bulls each. The levels of vitamin A intake and age when placed on the experiment are shown in table 1. The calves were handled in a routine manner prior to being placed on experiment. During the experiment all bulls were fed beet pulp and a grain mixture containing 100 lb. ground white corn, 85 lb. ground oats, 45 lb. wheat bran, 30 lb. linseed oil meal, 45 lb. soybean oil meal, 1.5 lb. iodized salt, 1.5 lb. bone meal, 1.5 lb. pulverized limestone, and 0.5 lb. irradiated yeast. Vitamin A (concentrated liver oil) was supplied daily as outlined in the table.

The various groups were kept in separate pens and exercising lots; each bull was tied during feeding. Semen collection was made with the artificial vagina, using estrus females. Blood plasma vitamin A determinations were made at bimonthly intervals on bulls in trial II from February to December, 1946, using the method of Kimble (7); blood carotene, ascorbic acid, and hemoglobin also were determined, but these data will not be presented in

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TABLE 1

Daily vitamin A intake and ages of animals on experiment

Trial and group	Herd no.	Breed	Initial age	Interval on expt.	Vitamin A		Remarks
					Date	Intake	
			(days)	*(days)*		*(I.U. daily)*	
I—Control	B548	Hol.	105	501	7/9/44	100,000	Sold 8/10/45
I—Control	B550	Hol.	94	505	7/9/44	100,000	Slaughtered 8/24/45
I—Control	B558	Hol.	63	436	8/23/44	100,000	Sold 8/31/45
I—Control	P111	Jer.	44	432	7/9/44	100,000	Slaughtered 8/1/45
I—Control	P112	Jer.	44	60	7/9/44	100,000	Died—cause unknown 9/7/44
I—Low	B549	Hol.	103	698	7/9/44 12/14/44 3/9/45	2,000 4,000 6,000	Testicular biopsy 9/19/45 Slaughtered 2/26/46
I—Low	B555	Hol.	48	643	7/9/44 12/14/44 3/9/44 9/19/44	2,000 4,000 6,000 100,000	Testicular biopsy 9/19/45; slaughtered 2/26/46
I—Low	B557	Hol.	40	616	7/28/44	Same as B555	Same as B555
I—Low	B554	Guer.	56	446	7/9/44 12/14/44 3/9/44	2,000 4,000 6,000	Slaughtered 8/3/45
I—Low	B553	Guer.	58	202	7/9/44 12/14/44	2,000 4,000	Died: pneumonia 12/20/44

TABLE 1—(*Continued*)

Trial and group	Herd no.	Breed	Initial age	Interval on expt.	Vitamin A		Remarks
					Date	Intake	
II—Control	B564	Hol.	44	532	8/1/45	100,000	Slaughtered 12/12/46
II—Control	B563	Guer.	75	573	8/1/45	100,000	Slaughtered 12/12/46
II—Control	B576	Hol.	44	28	2/19/46	100,000	Died: rumenitis 3/19/46
II—Control	P120	Jer.	49	488	9/29/45	100,000	Slaughtered 12/12/46
II—Medium	B565	Hol.	42	525	8/16/45 3/5/46	6,000 16/lb.	Slaughtered 12/11/46
II—Medium	B569	Hol.	45	474	10/9/45 5/12/46	6,000 16/lb.	Slaughtered 12/11/46
II—Medium	B575	Hol.	50	347	2/19/46 6/1/46	6,000 16/lb.	Slaughtered 12/11/46
II—Medium	P118	Jer.	50	548	8/1/45 4/18/46	6,000 16/lb.	Slaughtered 12/11/46
II—Low	B566	Hol.	42	519	8/16/45 3/5/46	4,000 8/lb.	Slaughtered 12/6/46
II—Low	B570	Hol.	42	454	10/20/45 4/18/46	4,000 8/lb.	Slaughtered 12/6/46
II—Low	B573	Hol.	44	383	1/8/46 7/16/46	4,000 8/lb.	Slaughtered 12/12/46
II—Low	P119	Jer.	42	525	8/16/45 4/18/46	4,000 8/lb.	Slaughtered 12/12/46

detail. Growth rate was measured and observations made for the clinical symptoms of vitamin A deficiency.

RESULTS

Trial I. As shown in table 1, two groups of five bulls each were started on experiment during July and August, 1944. On September 7, 1944, a Jersey bull (P112) in the control group died (cause undetermined), and on December 20, 1944, a Guernsey bull (B553) in the low vitamin A group died of pneumonia. This left three Holsteins and one Guernsey in the low group, and three Holsteins and one Jersey in the control group. As shown in figure 1, the high group grew satisfactorily throughout the experiment.

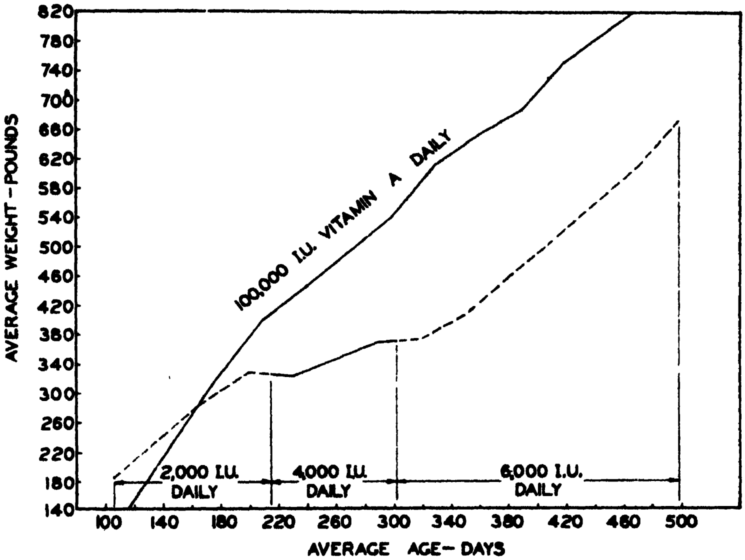

FIG. 1. Effect of vitamin A intake on rate of growth in dairy bull calves.

In December, 1944, bulls in the low group receiving 2,000 I.U. vitamin A daily began to lose weight, showing incoordination and reduced feed intake. By December 14, three of the four bulls in the low group were blind, and the vitamin A intake of all bulls in the group was raised to 4,000 I.U. daily. The bulls slowly recovered and gained at a low rate. The bull (B557) which had not gone blind while on 2,000 I.U. of vitamin A daily showed distinct blindness on February 23, 1945, while on 4,000 I.U. On March 9, 1945, all bulls were raised to 6,000 I.U. vitamin A daily (approximately 14 I.U. per lb. of weight). As body weight increased, the daily vitamin A intake decreased, until by September, 1945, it was about 9 I.U. per lb.

Satisfactory semen was produced by the bulls in the control group at 16 months of age, but only slight spermatogenesis was noted in bulls on the low-level intake. Two bulls from the control group (B550, P111) and one bull from the low group (B554) were slaughtered and autopsied during August, 1945. Histological sections were made of the vital organs, endocrine glands, and reproductive tracts. No gross pathology was evident in the bulls from the control group and only blindness in the bull from the low group. Histological examination of the testes revealed a moderate rate of spermatogenesis in all three bulls.

Testicular biopsies (2) were made on the three remaining Holstein bulls (B549, B555, B557) on September 19, 1945. Histological sections of the testes revealed retarded proliferation of the germinal epithelium, indicating delayed sexual maturity. Two of these bulls (B555, B557) then were raised to 100,000 I.U. of vitamin A daily while the other (B549) remained on 6,000 I.U. Although these bulls were blind, they gradually became accustomed to mounting cows in heat. Twelve samples of semen were collected from each bull during January and February, 1946. In only one sample (B549) was initial motility rated above 60 per cent. The concentration of sperm generally was below 500,000 per $mm.^3$ and survival time at 5° C. usually was less than 24 hours. The sex desire of these bulls was quite good. B549 (6,000 I.U. daily) consistently produced the best quality of semen, while the semen of B557 was of very inferior quality. These bulls were slaughtered and autopsied February 26, 1946. All three had cystic pituitaries. B555 had the largest cyst (approximately 2.5 cm. in diameter) and also showed gross kidney damage; histological examination of the kidneys revealed extensive albuminous degeneration of the convoluted tubules, leucocytic infiltration, and glomerular damage. The kidneys of B549 and B557 showed only very slight albuminous degeneration. The testes of these bulls showed a moderate rate of spermatogenesis, with germinal elements arranged fairly densely. The epididymis contained moderate numbers of stored sperm.

Trial II. This trial was started in August, 1945. The animals were placed on experiment as they became available. The details are shown in table 1. Bulls in the medium group received 6,000 I.U. vitamin daily until they reached 300 lb. in weight. The vitamin A intake then was adjusted to 16 I.U. per lb. daily. The low group received 4,000 I.U. daily until they reached 300 lb. (B566 until 400 lb.); intake then was adjusted to 8 I.U. per lb. daily. All bulls were weighed on 3 consecutive days each month and at such other times as necessary to regulate the vitamin A intake. These vitamin levels were selected because it was believed that 8 I.U. per lb. would be marginal and 16 I.U. per lb. would be adequate. The average intake of vitamin A per pound of body weight and rate of gain for each weight period for each bull used in this trial are shown in table 2. None of the bulls with the exception of B575 in the medium group grew as rapidly as the control bulls in trial I (fig. 1). This failure to duplicate growth rate was due partially to not attempting to feed for maximum growth, especially prior to March, 1946. The controls in this experiment (trial II) showed unsatisfactory

TABLE 2

Growth rate and daily vitamin A intake

Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)	Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)	Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)
B566 (Low group)					B565 (Medium group)					B564 (High group)				
Birth	0	92	...	...	Birth	0	87	...	...	Birth	0	84	...	
8-16-45	42	125	...	0.79	8-16-45	42	101	.	0.33	8-1-45	44	98	.	0.32
9-20-45	35	168	27.3	1.28	9-20-45	35	135	50.8	0.97	9-2-45	32	134	862.0	1.12
10-24-45	34	195	22.0	0.79	10-24-45	34	172	39.1	1.09	10-13-45	41	193	611.6	1.44
12-1-45	38	263	17.5	1.79	12-1-45	38	224	30.3	1.37	11-1-45	19	215	490.2	1.16
1-1-46	31	301	14.2	1.23	1-1-46	31	231	26.4	0.23	12-1-45	30	242	437.6	0.90
2-1-46	31	345	12.4	1.42	2-1-46	31	268	24.0	1.19	1-1-46	31	266	393.7	0.77
3-5-46	32	398	10.8	1.34	3-5-46	32	298	21.2	0.94	2-1-46	31	292	358.4	0.84
3-29-46	24	410	8.4	0.50	3-29-46	22	311	17.1	0.59	3-1-46	28	311	331.7	0.68
4-30-46	33	463	7.8	1.61	4-30-46	32	353	15.7	1.31	3-29-46	28	337	308.6	0.93
5-31-46	31	503	7.9	1.29	5-31-46	31	392	16.1	1.26	4-30-46	32	393	274.0	1.75
6-30-46	30	543	8.1	1.33	6-30-46	30	415	16.9	0.77	5-31-46	31	402	251.6	0.29
7-31-46	31	560	8.2	0.74	7-31-46	31	468	15.4	1.71	6-30-46	30	449	235.0	1.57
8-31-46	31	590	8.0	0.97	8-31-46	31	519	16.3	1.65	7-31-46	31	500	210.7	1.65
10-1-46	31	622	8.1	1.03	10-1-46	31	595	15.9	2.45	8-31-46	31	529	194.4	0.94
10-31-46	30	667	8.0	1.50	10-31-46	30	648	16.1	1.77	10-1-46	31	559	183.8	0.97
12-5-46	35	700	7.9	0.94	12-11-46	41	711	16.1	1.54	10-31-46	30	625	168.9	2.20
										12-11-46	41	673	154.1	1.17
B570 (Low group)					B569 (Medium group)					B563 (High group)				
Birth	0	86	...	...	Birth	0	95		.	Birth	0	61	...	...
10-20-45	42	100	.	0.33	10-9-45	45	97	. .	0.04	8-1-45	75	122	...	0.81
10-30-45	10	105	39.0	0.50	11-1-45	23	118	55.8	0.91	9-2-45	32	147	743.5	0.78
12-1-45	32	132	33.8	0.84	12-1-45	30	142	46.2	0.80	10-13-45	41	201	574.7	1.32
1-1-46	31	170	26.5	1.23	1-1-46	31	165	39.1	0.74	11-1-45	19	219	476.2	0.95
2-1-46	31	222	20.4	1.68	2-1-46	31	204	32.5	1.26	12-1-45	30	238	437.6	0.63
3-1-46	28	263	16.5	1.46	3-1-46	28	230	27.6	0.93	1-1-46	31	257	404.0	0.61
4-18-46	48	300	14.2	0.77	3-29-46	28	252	24.9	0.79	2-1-46	31	270	379.5	0.42
4-30-46	12	312	8.5	1.00	5-12-46	44	301	21.7	1.11	3-1-46	28	296	353.4	0.93
5-31-46	31	357	8.0	1.45	5-31-46	19	321	16.7	1.05	3-29-46	28	326	321.5	1.07
6-30-46	30	396	8.0	1.30	6-30-46	30	348	15.5	0.90	4-30-46	32	355	293.7	0.91
7-31-46	31	443	8.1	1.52	7-31-46	31	377	16.6	0.94	5-31-46	31	404	263.5	1.58
8-31-46	31	495	8.1	1.68	8-31-46	31	431	16.0	1.74	6-30-46	30	441	236.7	1.23
10-1-46	31	545	8.1	1.61	10-1-46	31	475	16.0	1.42	7-31-46	31	486	215.7	1.45
10-31-46	30	575	8.2	1.00	10-31-46	30	504	16.1	0.97	8-31-46	31	532	196.5	1.48
12-5-46	35	566	8.1	−0.26	12-11-46	41	523	16.4	0.46	10-1-46	31	588	178.6	1.81
										10-31-46	30	635	163.5	1.57
										12-11-46	41	583	164.2	−1.27

TABLE 2—(*Continued*)

Growth rate and daily vitamin A intake

Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)	Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)	Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)
B573 (Low group)					B575 (Medium group)									
Birth	0	96	. .		Birth	0	99							
1–8–46	44	111		0.34	2–19–46	50	156		1.12					
3–1–46	52	154	30.2	0.83	3–29–46	38	187	35.0	0.82					
3–29–46	28	177	24.2	0.82	4–30–46	32	243	27.9	1.75					
4–30–46	32	210	20.7	1.03	5–31–46	31	302	22.0	1.90					
5–31–46	31	251	17.4	1.32	6–30–46	30	353	15.9	1.70					
6–30–46	30	285	14.9	1.13	7–31–46	31	405	15.8	1.68					
7–16–46	16	302	13.6	1.06	8–31–46	31	477	15.4	2.32					
7–31–46	15	327	8.3	1.67	10–1–46	31	547	15.7	2.26					
8–31–46	31	379	7.8	1.68	10–31–46	30	605	16.0	1.93					
10–1–46	31	441	8.0	2.00	12–11–46	41	682	15.8	1.88					
10–31–46	30	473	8.3	1.07										
12–11–46	41	531	7.7	1.41										
P119 (Low group)					P118 (Medium group)					P120 (High group)				
Birth	0	51	. .	..	Birth	0	54	... ...		Birth	0	62		
8–16–45	42	66		0.36	8–1–45	50	73		0.38	9–29–45	49	73		0.22
9–20–45	35	96	49.4	0.86	9–2–45	32	104	67.8	0.97	11–1–45	33	100	1156.0	0.82
10–24–45	34	121	36.9	0.74	10–13–45	41	153	46.7	1.20	12–1–45	30	130	869.6	1.00
12–1–45	38	156	28.9	0.92	12–1–45	49	204	33.6	1.04	1–1–46	31	151	711.7	0.68
1–1–46	28	193	22.3	1.19	1–1–46	31	216	28.6	0.39	2–1–46	31	173	617.3	0.71
2–1–46	31	235	18.7	1.35	2–1–46	31	242	26.2	0.84	3–1–46	28	188	554.0	0.54
3–1–46	28	266	16.0	1.11	3–1–46	28	246	24.6	0.14	3–29–46	28	203	511.5	0.54
4–18–46	48	309	13.9	0.90	3–29–46	28	267	23.4	0.75	4–30–46	32	209	485.4	0.19
4–30–46	12	324	8.2	1.25	4–18–46	20	301	21.1	1.70	5–31–46	31	249	436.7	1.29
5–31–46	31	354	7.9	0.97	4–30–46	12	316	16.9	1.25	6–30–46	30	296	367.0	1.57
6–30–46	30	367	8.3	0.43	5–31–46	31	364	15.8	1.55	7–31–46	31	343	313.0	1.52
7–31–46	31	401	7.8	1.10	6–30–46	30	394	15.8	1.00	8–31–46	31	404	267.7	1.97
8–31–46	31	448	8.0	1.52	7–31–46	31	449	16.1	1.77	10–1–46	31	470	228.8	2.13
10–1–46	31	507	8.1	1.90	8–31–46	31	507	15.9	1.87	10–31–46	30	520	202.0	1.67
10–31–46	30	547	8.0	1.33	10–1–46	31	557	16.2	1.61	12–11–46	41	593	179.7	1.78
12–11–46	41	614	7.9	1.63	10–31–46	30	594	16.0	1.23					
					12–11–46	41	632	16.3	0.93					

growth rates and were off feed more times than the bulls in the other two groups (table 3). Between-group comparisons are not possible since the control group contained a Holstein, Guernsey, and Jersey, whereas the other two groups were composed of three Holsteins and one Jersey. Bulls in both the low and medium groups showed night blindness during the experiment (table 3); all except P119 (low group) had convulsions at one time or another, with B566 and B570 having the most serious and most frequent attacks; three bulls, B566 and B570 (low group), and B118 (medium group) went blind. All animals in the low and medium groups showed considerable papillary edema, and retinal hemorrhage was observed in three cases (P118, B566, B570). No other clinical symptoms of vitamin A deficiency were observed. The animals remained on feed and grew as well as the controls (table 2). Their hair coats had a fine, sleek appearance and the hides were pliable. No swelling was observed in the feet or legs.

Beginning in February, 1946, blood analyses for vitamin A, carotene, hemoglobin, and ascorbic acid were made bimonthly. Blood plasma carotene was of no consequence since these animals were receiving vitamin A. The hemoglobin increased slowly with increasing age, with no observable differences between groups, and whole blood and blood plasma ascorbic acid were not appreciably different between the groups. The results of blood plasma vitamin A analyses are shown in table 4. Bulls in the medium and low groups showed essentially the same low concentration of vitamin A in the blood plasma, which is further indication of suboptimum vitamin A intake. The controls were considerably higher. Boyer *et al.* (1) reported that blood plasma vitamin A levels of 10 γ or more per 100 c.c. are necessary for adequate vitamin A nutrition of the growing calf and that plasma values of 7–8 γ are borderline.

Semen collections were attempted on all bulls beginning at 13 months of age. Ten or more attempts were made with each animal. Although each bull was observed riding others, only the Guernsey control (B563) produced semen of high quality. Very few sperm were observed in the ejaculates from B564 (control) and B570 (low), and these showed only slight motility. The other bulls that ejaculated (B573, B565, B575) produced no sperm. The ages at first ejaculation are shown in table 3. The bulls from which no ejaculates were obtained showed no interest in estrus cows at collection time.

Since bulls in the low and medium groups all showed various stages of vitamin A deficiency during November, 1946, all bulls were slaughtered during the following month. The vital organs were weighed and histological sections made. Organ weights and percentages of body weight are shown in table 5. The results of histological examination of the testes are shown in table 6. The organs and glands not otherwise described appeared histologically normal.

No pituitary cysts developed in the five control bulls which were autopsied

TABLE 3

Observations of bulls in trial II

Group	Bull	Days on experiment when observed					Age at 1st ejaculation	Remarks
		Off feed	Scouring	Convulsions	Night blindness	Blindness		
		(days)	*(days)*	*(days)*	*(days)*	*(days)*	*(days)*	
Low	B566			392	352	430		
	B570	359, 375, 401		329	263	390	449	Slight pneumonia 11/15/45
	B573			318	248		377	
	P119	326			454			
Medium	B565			457	352		432	
	B569	370, 386	310 330	400	293			Scoured considerably after 8/46
	B575			294	207		340	
	P118	412		457	352	388		Hoof rot 7/10/46
Control	B564	446					495	
	B563	249, 443, 454					407	Pneumonia 11/3/46
	P120	182, 206, 274 382						Hoof rot 11/19/46

TABLE 4

Micrograms of vitamin A per ml. of blood plasma

Date* (1946)	Low group				Medium group				High group		
	B566	B570	B573	P119	B565	B569	B575	P118	B564	B563	P120
Mar. 8	0.23	0.18	0.18	0.16	0.14	0.14	0.17	0.16	0.27	0.32	0.35
April 18	0.16	0.13	0.10	0.13	0.13	0.13	0.19	0.16	0.23	0.34	0.30
May 31	0.13	0.12	0.11	0.22	0.17	0.13	0.19	0.18	0.39	0.38	0.37
July 11	0.12	0.20	0.17	0.23	0.19	0.11	0.12	0.14	0.32	0.36	0.41
Aug. 24	0.12	0.11	0.12	0.15	0.13	0.07	0.12	0.14	0.39	0.45	0.52
Oct. 4	0.10	0.11	0.12	0.23	0.20	0.15	0.17	0.22	0.43	0.57	0.50
Nov. 14	0.09	0.08	0.10	0.12	0.11	0.06	0.07	0.10	0.31	0.33	0.40
Dec. 12†	0.05	0.05	0.04	0.10	0.06	0.04	0.04	0.10	0.31	0.30	0.46

* Av. of three determinations made on the date, 2 weeks prior, and 2 weeks after.

† Av. of three consecutive daily samples taken prior to slaughter.

TABLE 5

Organ weights and percentage of body weight

Organ	Wt.	% body wt.	Wt.	% body wt.	Wt.	% body wt.	Wt.	% body wt.
	B566		B570		B573		P119	
Low group								
Testes (g.)	410.9	0.129	350.5	0.137	186.0	0.077	278.1	0.100
Epid. (g.)	40.9	0.013	26.7	0.010	15.6	0.006	31.3	0.011
S. vesc. (g.)	40.4	0.013	34.9	0.014	38.0	0.016	43.7	0.016
Cowpers (g.)	4.1	0.001	3.1	0.001	3.1	0.001	4.4	0.002
Pit. (g.)*	1.6	0.0005	2.0	0.0008	1.5	0.0006	1.5	0.0006
Thyroid (g.)	22.2	0.007	23.8	0.009	16.2	0.007	13.2	0.005
Adr. (g.)	16.3	0.005	6.8	0.003	11.0	0.005	12.2	0.004
	B565		B569		B575		P118	
Medium group								
Testes (g.)	240.3	0.074	201.8	0.085	248.0	0.082	414.3	0.144
Epid. (g.)	46.6	0.014	18.2	0.008	20.6	0.007	32.6	0.011
S. vesc. (g.)	75.8	0.024	44.9	0.019	62.9	0.020	40.5	0.014
Cowpers (g.)	4.3	0.001	3.5	0.002	4.7	0.002	4.8	0.002
Pit. (g.)*	2.2	0.0007	1.6	0.0007	1.7	0.0005	1.9	0.0007
Thyroid (g.)	23.3	0.007	18.4	0.008	35.7	0.012	19.9	0.007
Adr. (g.)	15.8	0.005	10.9	0.005	11.4	0.004	13.4	0.005
	B564		B563				P120	
Control group								
Testes (g.)	264.9	0.087	344.4	0.130			360.6	0.134
Epid. (g.)	36.3	0.012	37.8	0.014			24.6	0.009
S. vesc. (g.)	48.6	0.016	59.0	0.022			39.5	0.015
Cowpers (g.)	3.6	0.001	4.5	0.002			5.0	0.002
Pit. (g.)*	1.7	0.0006	1.9	0.0007			1.8	0.0007
Thyroid (g.)	23.3	0.008	22.2	0.008			15.4	0.006
Adr. (g.)	11.9	0.004	19.0	0.007			14.4	0.005

* Whole pituitary minus cyst.

in trials I and II. Edema and vacuolation of the basophiles were observed in B550 and P120. Seven of the nine bulls receiving the low level of vitamin A developed pituitary cysts ranging in diameter from 0.5 to 3 cm. The pituitary histology of the bulls without cysts (B553 and B554) was relatively normal. The remainder of the pituitaries of this group were characterized by a loose arrangement of the anterior pituitary cells and vacuolation of the basophiles, and edema was observed in five of the glands. All of the four bulls in the medium vitamin A group in trial II developed pituitary, cysts. Two of the bulls (B565 and B569) showed some edema, vacuolation of the basophiles, and a loose arrangement of the pituitary elements.

TABLE 6

Trial II—histological observations on testes and epididymides

Group	Bull no.	Testes	Epididymides
Low	B566	Low rate of spermatogenesis. Some tubules contained only spermatogonia.	Left: few stored sperm, some leucocytic infiltration; right: large number of stored sperm.
Low	B570	Very low rate of spermatogenesis. Primary and secondary spermatocytes only in most tubules.	Moderate numbers of sperm.
Low	B573	No sperm. Most tubules contained only spermatogonia and primary spermatocytes.	No sperm.
Low	P119	Low rate of spermatogenesis. Some tubules with no lumina and containing only spermatogonia and a few primary spermatocytes.	Abundant stored sperm.
Medium	B565	Very low rate of spermatogenesis. Some tubules with no lumina and containing only spermatogonia and a few primary spermatocytes.	No sperm.
Medium	B569	An occasional sperm. Most tubules had primary and secondary spermatocytes only.	Epithelial cells in various stages of mitosis and a few sperm heads.
Medium	B575	No sperm. Most tubules contained spermatogonia and secondary spermatocytes, and a few spermatids.	No sperm. Few epithelial cells.
Medium	P118	Low rate of spermatogenesis. Some tubules with primary spermatocytes and spermatogonia only.	Large numbers of sperm.
Control	B564	Low rate of spermatogenesis.	Left: numerous areas with leucocytic infiltration, moderate sperm storage; right: abundant sperm.
Control	B563	Moderate spermatogenesis.	Abundant sperm.
Control	P120	Moderate spermatogenesis.	Abundant sperm.

No consistent differences occurred between groups in the histology of the adrenal cortex but some variation from normal appeared in the adrenal medulla. In one of the control bulls (B550) slight necrobiosis of the medulla was observed, and in a second control (B564) there was moderate lymphocytic infiltration of the medulla. In five of the nine bulls on low vitamin A intake early necrobiosis or necrosis of the medullary cells was observed and in one (B555) several large areas of polymorphonuclear leucocytes extended from the capsule through the medullary portion. In the medium vitamin A group early necrobiosis was observed in B565 and B569, the bulls with the largest pituitary cysts in the group. One area of the cortex was infiltrated with polymorphonuclear leucocytes in P118.

DISCUSSION

There is general agreement among investigators that vitamin A deficiency, if prolonged, results in the degeneration of the germinal epithelium of the testes in sexually mature bulls and that lowered fertility or sterility results. It is likewise agreed, that in sexually mature bulls, relatively normal spermatogenesis can be restored by vitamin A therapy (2, 3, 4, 5, 9, 12). The effects of prepubertal vitamin A deficiency upon sexual development and subsequent fertility or reproductive capacity are not completely understood. Following the reports of Madsen *et al.* (10) that prenatal or postnatal vitamin A deficiency in cattle results in the development of pituitary cysts which are not reparable by vitamin A therapy, it was hypothesized that suboptimal vitamin A intake prior to puberty might be a cause of lowered fertility in mature bulls.

It originally was intended that the level of vitamin A intake in the Purdue experiment would prevent clinical vitamin A deficiency and that the results of suboptimal vitamin A could be measured for the most part in terms of semen quality. As reported above, the results were not wholly as expected. In the first experiment, three Holstein bulls on low vitamin A intake became blind by 6 months of age. The development of blindness resulted in continual difficulty in making semen collections, and because of this complication, it is impossible to separate the physiological and psychological effects of vitamin A deficiency on reproductive capacity. However, the microscopic study of testicular biopsies made on the three bulls at 14 months of age showed that spermatogenesis was retarded. Two of the animals received 100,000 I.U. of vitamin A daily beginning at the fourteenth month and one was held at 6,000 I.U. Although blindness persisted, all of the bulls eventually ejaculated. None of the semen samples could be classified as of excellent quality, although large numbers of sperm were produced. Examination of the testes at autopsy revealed that the rate of spermatogenesis was suboptimum in terms of bulls of known high fertility. Evidences of pituitary and adrenal damage were observed. Although the authors are not specialists in pituitary or adrenal cytology, certain histo-pathological changes were noted.

Pituitary cysts were present in the three bulls. The cells of the anterior pituitaries were loosely arranged; there were evidences of edema, and the basophiles showed more vacuolation than in normal bulls. Clear-cut castration cells were not observed. Although vitamin A therapy was carried out for 5 months, spermatogenesis was not restored to an entirely satisfactory level and histological evidences of vitamin A deficiency persisted. These results are in essential agreement with those of Madsen *et al.* (10) and Hodgson *et al.* (5). It would appear, however, that acute vitamin A deficiency in sexually immature animals may have effects which persist long after the correction of the deficiency.

The second experiment was planned with the intention of studying both vitamin A deficiency and subclinical deficiency in prepubertal bulls. Vitamin A levels of 4,000 and 6,000 I.U. per day until the bulls reached 300 lb., and adjustment at that time to 8 and 16 I.U. of vitamin A per lb. body weight, were maintained. These levels are equivalent to 17.6 and 35.3 I.U. per kg. of body weight and are approximately one-half and slightly above the minimum requirements (32 I.U. per kg.) for calves as reported by Lewis and Wilson (8). As previously pointed out, neither the control nor experimental bulls in trial II grew as rapidly as the controls in trial I. This difference apparently is the result of a lower daily feed intake than was maintained in the first experiment. All of the bulls in the low and medium vitamin A groups developed night blindness and all except one (P119—low group) had convulsions during the trial. Papillary edema and retinal hemorrhage were observed in three bulls, and blindness developed in three cases. However, other clinical evidences of vitamin A deficiency were not observed. Growth rate of the bulls in the low and medium groups was essentially similar to that of the controls receiving 100,000 I.U. of vitamin A daily, and the hides were sleek and pliable. Normal sexual maturity, as measured by sexual drive and semen quantity and quality, was retarded in the low and medium groups.

Histological studies of the entire reproductive tract and the vital organs were made. The rate of spermatogenesis in eight of the experimental and one of the control bulls was low and definitely suboptimal. Sperm production was reduced.

Although Hodgson *et al.* (5) demonstrated fertility in the semen of vitamin A-deficient bulls, they found that semen quality as measured by the usual standards was low. It is the opinion of the authors that although bulls receiving deficient or slightly deficient rations may not be sterile, fertility is affected and reproductive capacity definitely is limited. The reduction of sexual drive and failure to ejaculate are serious reproductive disturbances. The ability of males with these characteristics to impregnate an occasional female is an indication of fertility but is no measure of normal reproductive capacity.

The development of recognizable pituitary and adrenal changes in the bulls receiving 8 and 16 I.U. of vitamin A suggests that these intakes do not permit complete physiological development, even though growth may be relatively satisfactory.

The results which have been obtained in these and other studies on the vitamin A requirements of growing bulls leave much to be desired. Variations between bulls of the same and different breeds, failure to adjust vitamin A intake to body weight from birth, and the failure to conduct blood vitamin A studies beginning at birth all have contributed to the difficulty of reaching entirely satisfactory conclusions in this study. The interference of blindness with normal behavior may account for some of the observations on the apparent lack of sexual drive, but there seems to be sufficient evidence to suggest that suboptimal vitamin A intake retards male hormone production as well as spermatogenesis.

SUMMARY

1. The effects of varying levels of vitamin A on the reproductive development of 13 Holstein, 4 Jersey, and 3 Guernsey bulls were studied.

2. Complete blindness developed in bulls receiving 2,000 I.U. of vitamin A daily. Following supplementation of the ration with 4,000 and 6,000 I.U. of vitamin A daily, very slight spermatogenesis was observed; only moderate sperm production was found following 5 months of therapy with 100,000 I.U. of vitamin A daily.

3. Bulls which received 4,000 and 6,000 I.U. daily of vitamin A until 300 lb. in weight, and then 8 and 16 I.U. daily per lb. of body weight, exhibited convulsions, showed evidence of night blindness, and three became totally blind. Sexual maturity was delayed, sex drive inhibited, and spermatogenesis limited. Cystic pituitary glands were observed in all bulls except the controls. Histological differences were observed, however, in both the anterior pituitary glands and the adrenal glands of the bulls.

4. Although moderate prepubertal vitamin A deficiency did not completely inhibit sperm production, fertility and reproductive capacity appeared to be seriously impaired, since sexual drive and spermatogenesis as measured by laboratory procedures and histological examination were reduced. The possibility that prepubertal damage may be permanent, especially when cystic pituitaries develop or total blindness occurs, should not be overlooked.

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THE EFFECT OF MILKING AT SHORT INTERVALS WITH AND WITHOUT INJECTIONS OF OXYTOCIN[1]

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Kellner (7) long ago demonstrated an increased production of milk per unit of time by decreasing the milking interval. Many methods since have been used to ascertain the course of milk secretion. Tgetgel (10) measured the increase in pressure within the mammary gland during the intervals between milkings. His data show that the pressure increases slowly after milking, but rises quite rapidly toward the approach of the next milking. Isaachsen (6) attempted to measure the course of milk secretion by canulating the mammary gland over a 24-hour period. The container into which the catheter drained was emptied at hourly intervals. The flow of milk from hour to hour was quite variable.

Filipovic (2) milked cows at one-half and hour intervals during the periods between normal milkings. The yields that were obtained were quite irregular. He interpreted the data as showing a peak secretion occurring at about 4 hours after the last normal milking.

Ragsdale *et al.* (8) and Garrison and Turner (4) studied the rate of milk secretion by milking cows at increasing intervals of from 1 to 36 and 1 to 24 hours, respectively. These authors concluded that the rate of milk secretion is quite constant at milking intervals of 12 hours.

Gasnier *et al.* (5) milked cows at intervals ranging from 0.5 to 16 hours. Their conclusions were that milk production was proportional to the length of the interval during the first 6 hours, and for longer intervals milk production varied logarithmically.

Shaw (9), milking cows at 2-hour intervals with the aid of oxytocin, found that milk secretion was uniform from milking to milking. According to the theory proposed by Ely and Petersen (1), it is necessary for the cow to respond to the stimulus of milking by releasing the hormone, oxytocin, thereby causing the "let-down" phenomenon before the preformed milk in the cow's udder can be removed.

EXPERIMENTAL METHODS

Eight Holstein cows were selected from the University herd, on the basis of level of production and stage of lactation, to form four pairs such that the two individuals of a pair were as nearly alike as possible.

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Records of the milk weights and fat tests on each cow were kept for a 6-day pre-experimental period. During the subsequent 6 days, or experimental period, the cows were milked 12 times in 24 hours, beginning at 6 a.m. Thus the 6 a.m. milking constituted the beginning of a new day. On the injection day for a given group, each cow of the group was injected with 10 I.U. of oxytocin immediately prior to each milking.

The design of the experimental period is illustrated as follows:

Day of experimental period	1	2	3	4	5	6
Group I	Pre-injection	Injection	Inter-injection	Injection	Post-injection	Post-injection
Group II	Pre-injection	Pre-injection	Injection	Inter-injection	Injection	Post-injection

Days 2, 3, 4, and 5 of the experimental period constituted a "switchback" design with respect to injection. However, the results obtained from cows of Group I on the sixth day of the experimental period have no counterparts among the results obtained from the cows of Group II. Also, the results from the cows of Group II have no counterparts obtained from Group I on the shift from twice-a-day to 12-times-a-day milking on the first day of the experimental period.

Records were kept of the pounds of milk produced and the per cent of fat of the milk for each cow at each of the 12 daily milkings during the 6 days of the experimental period.

The cows were returned to their regular twice-a-day milking schedule following the 6-day experimental period. Records again were kept of the pounds of milk and per cent of fat for each cow at each milking for 7 days subsequent to the experimental period. The day immediately after the experimental period was considered a transitional period, and the next 6 consecutive days were termed the post-experimental period.

One of the necessary conditions for valid use of analysis-of-variance methods is uniform variability of all the measurements involved in the analysis. The tests of significance associated with analysis-of-variance procedures are meaningless when this condition is not fulfilled, at least approximately. Since data for the respective cows differ in variability, and the variability is greatly reduced during the period of injection and greatly increases after cessation of injection, the data were analyzed by the rank method (3), as no common transformation would "bring the data into line".

RESULTS

Comparison of the Pre-experimental, Experimental and Post-experimental Periods

Average daily production of milk. Table 1 gives the 6-day averages of the milk production of each cow for each of the three 6-day periods involved

TABLE 1

Average daily production of milk by each cow during each of the three periods of the study

Group	Cow	Period					
		Pre-experimental		Experimental		Post-experimental	
		lb.	Rank	lb.	Rank	lb.	Rank
I	228	36.3	2	42.6	3	34.0	1
	282	38.6	2	43.8	3	37.6	1
	290	28.7	2	32.9	3	27.6	1
	190	11.9	2	16.8	3	6.6	1
II	242	41.0	2	49.2	3	37.7	1
	258	41.6	2	48.2	3	36.0	1
	307	26.1	2	30.2	3	24.5	1
	174	13.6	2	17.1	3	12.8	1
Av. standard error		0.44		1.16		0.52	

in the study. The averages for each cow were ranked 1, 2, or 3 in increasing order of magnitude. The most conspicuous feature of the results is the complete consistency of the ranks, as the average daily production of every cow was greatest during the experimental period and least during the post-experimental period. Since the probability of eight sets of three ranks each being completely consistent by chance alone is 1/279,936, it is clear that the particular pattern of complete consistency observed is significant. The average standard error shown in table 1 for the experimental period is more than double the values shown for each of the other two periods. This increase in variability, which is significant at the 0.01 probability level, largely is due to the vicissitudes of milk production on the days following injection days, which greatly over-balanced the greater uniformity of milk production on injection days.

TABLE 2

Weighted average per cent fat of milk produced by each cow during each of the three periods of the study

Group	Cow	Period					
		Pre-experimental		Experimental		Post-experimental	
		%	Rank	%	Rank	%	Rank
I	228	3.51	2	3.37	1	3.85	3
	282	3.77	2	3.54	1	4.26	3
	290	3.64	2	3.48	1	4.05	3
	190	4.66	2	3.75	1	5.04	3
II	242	3.57	2	3.31	1	4.10	3
	258	3.48	2	3.25	1	4.10	3
	307	3.89	2	3.63	1	4.78	3
	174	3.34	1	3.40	2	3.59	3
Av. standard error		0.069		0.121		0.173	

Average per cent fat. The average per cent of fat for each period is presented in table 2, together with the relative ranks. These are weighted averages of the figures on per cent of fat for the respective milkings and are not simple arithmetic means.

For each cow the highest average per cent fat occurred during the post-experimental period, in which the average daily production of milk was least. Also, with the exception of cow no. 174, the lowest average per cent of fat occurred during the experimental period, when the average daily production of milk was greatest. Since the probability of obtaining by chance alone either a perfect negative correlation between two groups of eight sets of three ranks each or a case only one permutation distance from a perfect negative correlation is 1/559,872, the observed negative correlation is statistically significant. Average standard errors shown at the bottom of table 2 increase uniformly from the pre-experimental period through the post-experimental period. This trend is significant at the 0.01 probability level. From these data it is not possible to determine whether this is due to injections and their after-effects, or to the effect of 12 milkings per day during the experimental period and the after-effects of this procedure, or both.

TABLE 3

Average daily production of fat by each cow during each of the three periods of the study

Group	Cow	Period					
		Pre-experimental		Experimental		Post-experimental	
		lb.	Rank	lb.	Rank	lb.	Rank
I	228	1.27	1	1.43	3	1.31	2
	282	1.46	1	1.55	2	1.60	3
	290	1.04	1	1.14	3	1.12	2
	190	0.55	2	0.63	3	0.33	1
II	242	1.46	1	1.63	3	1.55	2
	258	1.45	1	1.56	3	1.48	2
	307	1.02	1	1.09	2	1.17	3
	174	0.455	1	0.58	3	0.458	2
Av. standard error . .		0.1067		0.0762		0.0486	

Average daily production of fat. Table 3 gives the 6-day averages of the *fat production (in pounds) of each cow for each of the periods of the study, as well as the relative ranks of the three averages for each cow. The figures* for average daily production of fat do not form as clear-cut patterns as the figures shown in tables 1 and 2. However, for each cow the average daily production of fat was higher during the experimental period than during the pre-experimental period, and, with the exception of cow no. 190, it also was higher during the post-experimental period than during the pre-experimental period. Since the probability of obtaining by chance alone a pattern equally or more consistent is 2/2,187, the pattern observed may be regarded as significant. The average standard errors of total fat production shown at the bottom of table 3 decrease uniformly from the pre-experimental period through the post-experimental period, a trend that is significant at the 0.01 level and in the opposite direction from that observed in the average standard errors of per cent fat.

Effect of Injections

Level of total milk production. The total production of milk (in pounds) of each cow on each of the 12 daily milkings during the 6-day experimental period is shown in figure 1. Without exception, the milk production on the first milking of an injection day either equaled or exceeded the maximum production of the other milkings on the same day, and without exception the milk production on the first milking of a control day following an injection day was below average for the day, although not the minimum for the day in all cases. Consequently, the first two milkings of each day were excluded *from the analyses to eliminate the "transitional milkings" from* one treatment to the other.

TABLE 4

Milk production (exclusive of the two "transitional" milkings) of each cow for each day of the experimental period

Day	Type	Group I, cow no.:							
		228		282		290		190	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	30.0	1	35.3	3	25.4	1	11.3	1
2	Injection	35.1	4	37.5	4	26.8	2	12.7	2
3	Inter-injection	34.0	3	41.6	5	26.9	3	18.1	5
4	Injection	38.5	5	42.6	6	30.8	6	18.3	6
5	Post-injection	40.0	6	30.9	1	27.7	4	14.4	3
6	Post-injection	33.5	2	34.5	2	28.4	5	15.0	4
Day	Type	Group II, cow no.:							
		242		258		307		174	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	40.0	3	37.1	1	26.7	5	14.2	3
2	Pre-injection	43.5	5	39.6	3	26.4	4	14.9	5
3	Injection	42.5	4	40.1	4	25.6	3	14.5	4
4	Inter-injection	37.7	1	39.1	2	24.3	2	12.8	2
5	Injection	46.3	6	46.5	6	27.3	6	16.8	6
6	Post-injection	39.1	2	42.2	5	22.6	1	12.7	1

Table 4 gives the total production of milk of each cow for the last ten milkings of each day of the experimental period, and the relative rank of each day's production. The rankings for both groups exhibit significant divergence from chance arrangements, the divergence being significant at the

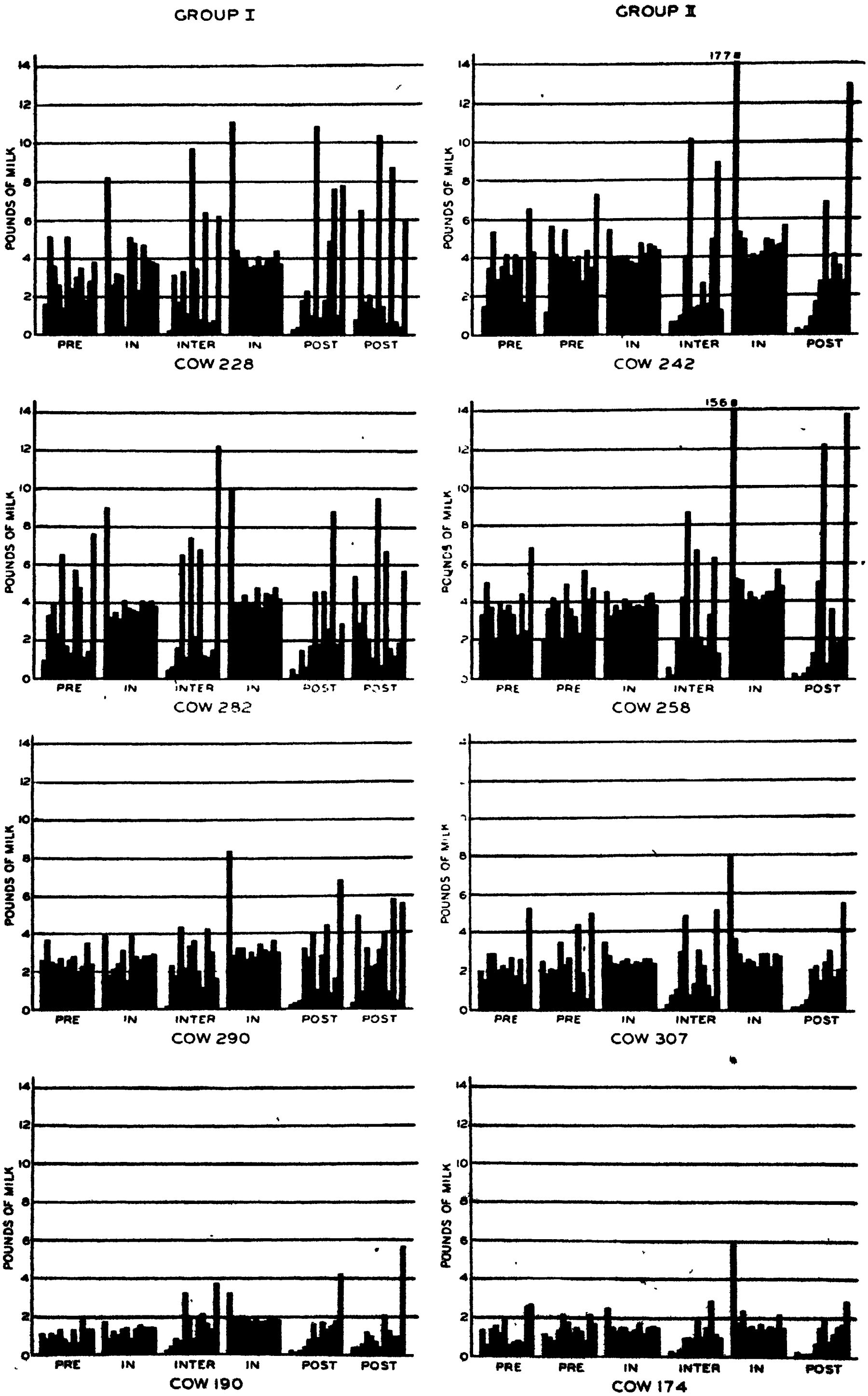

FIG. 1. Total milk (in lb.) of each cow on each of the twelve daily milkings on each of the 6 days of the experimental period.

0.05 level in the case of Group I and at the 0.01 level in the case of Group II. The only features common to the two parts of the table, however, appear to be the above-average production of the second injection day, and the fact that production on the second injection day exceeded production on the first injection day without exception.

Variability of total milk production. Examination of the various parts of figure 1 shows that, neglecting the initial milking on injection and post-injection days, milk production in general was more uniform from milking to milking on injection days than on pre-injection days, and generally more variable on post-injection days than on pre-injection or inter-injection days.

TABLE 5

Measures of the variability of the milk production from milking to milking (exclusive of the two "transitional" milkings) of each cow for each day of the experimental period*

Day	Type	Group I, cow no.:							
		228		282		290		190	
		lb.^{2}	Rank	lb.^{2}	Rank	lb.^{2}	Rank	lb.^{2}	Rank
1	Pre-injection	10.2	2	55.6	4	1.5	2	1.6	3
2	Injection	17.4	3	1.0	1	3.6	3	0.4	2
3	Inter-injection	86.6	4	132.5	6	11.7	4	9.2	4
4	Injection	0.6	1	1.3	2	0.8	1	0.1	1
5	Post-injection	116.0	5	51.4	3	34.6	6	11.2	5
6	Post-injection	120.1	6	78.3	5	32.1	5	21.5	6

Day	Type	Group II, cow no.:							
		242		258		307		174	
		lb.^{2}	Rank	lb.^{2}	Rank	lb.^{2}	Rank	lb.^{2}	Rank
1	Pre-injection	16.4	4	17.1	4	10.9	3	5.4	5
2	Pre-injection	13.9	3	11.4	3	17.8	4	2.2	3
3	Injection	1.2	1	0.7	1	0.1	1	0.2	1
4	Inter-injection	100.7	5	59.9	5	25.6	6	4.3	4
5	Injection	2.7	2	2.6	2	0.8	2	1.1	2
6	Post-injection	121.7	6	217.6	6	20.1	5	6.2	6

* Sums of the squares of the deviations of the pounds of milk of the 10 non-transitional milkings from the corresponding averages were used to indicate the variability in the pounds of milk.

Table 5 gives the value of the sum of squared deviations of the milk yields of each cow for the ten non-transitional milkings from the average yield of these milkings on the day concerned. The relative ranks of the six figures for each cow also are given. The measures of milking-to-milking variation of milk production clearly are consistent with the visual impression obtained from examination of figure 1; they are extremely small for injection days and extremely large for post-injection days. Without further statistical analysis, it is clear that injection results in a significant reduction in milking-to-milking variation of milk production and a significant increase in variability on days following injection.

Level of per cent of fat. The per cent fat of the milk produced by each

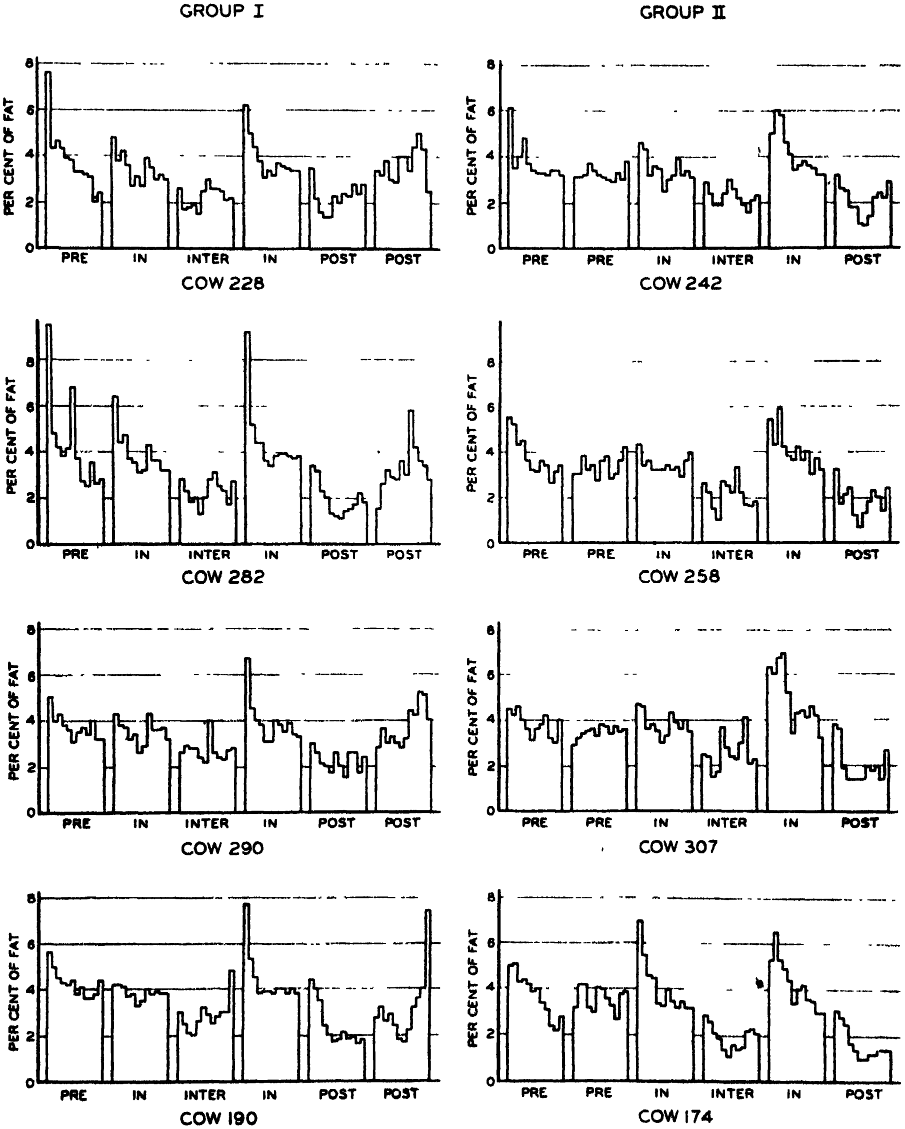

FIG. 2. The per cent fat of the milk of each cow on each of the twelve daily milkings on each of the 6 days of the experimental period.

cow on each of the 12 daily milkings during the 6-day experimental period is shown in figure 2. The per cent of fat of the milk produced on the first two (or three) milkings of an injection period generally was above average, with the per cent fat on the first injection milking generally the highest, except for cows of Group II on their second injection day. The per cent fat of milk produced on a day immediately following an injection day tends to be less than the per cent fat produced on either an injection day or on a pre-injection day.

Table 6 gives the per cent fat of the milk produced by each cow on the ten non-transitional milkings (*i.e.*, excluding the first two "transitional" milkings) of each day of the experimental period. The relative ranks of the six figures given for each cow also are shown. The pattern of ranks for the cows of Group I and Group II differs from random arrangements, to extents that are significant at the 0.01 probability level. A common feature of the rank patterns for these two groups of cows is the extreme below-average rank of the per cent fat produced on a day immediately following an injection day. A contrasting feature, common to both groups, is the above-average rank of per cent fat produced on the second injection day. Examination of graphs of per cent fat per milking for each cow on each day of the experimental period (fig. 2) indicates the presence of considerable variation in per cent fat from milking to milking within a day.

TABLE 6

Per cent of fat of milk produced (exclusive of the two "transitional" milkings) by each cow on each day of the experimental period

Day	Type	Group I, cow no.:							
		228		282		290		190	
		%	Rank	%	Rank	%	Rank	%	Rank
1	Pre-injection	3.40	4	3.41	3	3.57	4	4.06	5
2	Injection	3.28	3	3.59	4	3.43	3	3.78	3
3	Inter-injection	2.38	1	2.41	2	2.74	2	3.16	2
4	Injection	3.55	5	3.87	6	3.60	5	3.95	4
5	Post-injection	2.39	2	1.58	1	2.20	1	1.86	1
6	Post-injection	3.73	6	3.79	5	3.95	6	4.53	6
Day	Type	Group II, cow no.:							
		242		258		307		174	
		%	Rank	%	Rank	%	Rank	%	Rank
1	Pre-injection	3.55	5	3.43	5	8.79	5	3.36	3
2	Pre injection	3.32	4	3.41	4	3.54	3	3.59	4
3	Injection	3.27	3	3.35	3	3.65	4	3.63	5
4	Inter injection	2.15	2	2.06	2	2.66	2	1.80	2
5	Injection	3.87	6	3.93	6	4.51	6	3.97	6
6	Post-injection	2.08	1	1.75	1	1.91	1	1.28	1

Sums of the squares of the deviations of the per cent fat of the ten non-transitional milkings from the corresponding averages were used to indicate the variability in per cent of fat among the pre-injection, injection, and post-

TABLE 7

Measures of the variability of the per cent fat of the milk produced on each milking (exclusive of the two "transitional" milkings) by each cow on each day of the experimental period*

Day	Type	Group I, cow no.:							
		228		282		290		190	
		$\%^2$	Rank	$\%^2$	Rank	$\%^2$	Rank	$\%^2$	Rank
1	Pre-injection	5.77	6	14.52	6	1.32	2	1.06	3
2	Injection	2.25	3	2.45	3	2.04	4	0.48	2
3	Inter-injection	1.84	2	2.82	4	2.32	5	5.43	5
4	Injection	1.20	1	0.90	1	1.13	1	0.40	1
5	Post-injection	2.46	4	1.58	2	1.54	3	2.76	4
6	Post-injection	5.38	5	7.48	5	7.10	6	24.74	6
Day	Type	Group II, cow no.:							
		242		258		307		174	
		$\%^2$	Rank	$\%^2$	Rank	$\%^2$	Rank	$\%^2$	Rank
1	Pre-injection	2.22	4	2.90	3	2.37	4	5.90	6
2	Pre-injection	0.80	1	2.09	2	0.22	1	2.32	3
3	Injection	1.28	2	0.72	1	1.22	2	2.58	4
4	Inter-injection	1.49	3	4.02	5	6.15	5	1.52	1
5	Injection	5.72	6	5.72	6	8.70	6	5.42	5
6	Post-injection	3.50	5	2.96	4	1.66	3	1.70	2

* Sums of the squares of the deviations of the per cent fat of the 10 non-transitional milkings from the corresponding averages were used to indicate the variability in per cent of fat.

TABLE 8

Total fat produced by each cow each day (exclusive of the two "transitional" milkings) during the experimental period

Day	Type	Group I, cow no.:							
		228		282		290		190	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	1.02	3	1.20	3	0.91	3	0.46	2
2	Injection	1.15	4	1.35	5	0.92	4	0.48	3
3	Inter-injection	0.81	1	1.00	2	0.74	2	0.57	4
4	Injection	1.37	6	1.65	6	1.11	5	0.72	6
5	Post-injection	0.95	2	0.49	1	0.61	1	0.27	1
6	Post-injection	1.25	5	1.31	4	1.12	6	0.68	5
Day	Type	Group II, cow no.:							
		242		258		307		174	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	1.42	4	1.27	3	1.01	5	0.48	3
2	Pre-injection	1.44	5	1.35	5	0.94	4	0.535	5
3	Injection	1.39	3	1.34	4	0.93	3	0.527	4
4	Inter-injection	0.810	1	0.81	2	0.65	2	0.23	2
5	Injection	1.79	6	1.83	6	1.23	6	0.67	6
6	Post-injection	0.813	2	0.74	1	0.43	1	0.16	1

injection days. These measures of variation are presented in table 7 with the relative ranks of the six measures for each cow. The pattern of ranks for Group I and Group II differs from random arrangements to extents that are significant at the 0.01 and 0.05 probability levels, respectively.

Table 8 gives the total fat produced by each cow in the last ten milkings of each day of the experimental period, together with the relative ranks of the six figures given for each cow. The pattern of ranks of Groups I and II are very similar to the corresponding patterns for per cent fat given in table 6. Cessation of injections was followed by a slump in fat production, followed by a high on the second day after injection.

TABLE 9

Measures of the variability of the total fat produced on each milking (exclusive of the two "transitional" milkings) by each cow on each day of the experimental period*

Day	Type	Group I, cow no.:							
		228		282		290		190	
		$lb.^2$	Rank	$lb.^2$	Rank	$lb.^2$	Rank	$lb.^2$	Rank
1	Pre-injection	0.0198	3	0.0569	4	0.0022	1	0.0022	3
2	Injection	0.0165	2	0.0028	1	0.0050	3	0.0007	2
3	Inter-injection	0.0564	4	0.0980	5	0.0125	4	0.0204	5
4	Injection	0.0027	1	0.0036	2	0.0023	2	0.0002	1
5	Post-injection	0.0822	5	0.0138	3	0.0230	5	0.0034	4
6	Post-injection	0.2013	6	0.1549	6	0.0783	6	0.1408	6
Day	Type	Group II, cow no.:							
		242		258		307		174	
		$lb.^2$	Rank	$lb.^2$	Rank	$lb.^2$	Rank	$lb.^2$	Rank
1	Pre-injection	0.0212	3	0.0195	3	0.0215	4	0.00486	5
2	Pre-injection	0.0266	4	0.0156	2	0.0224	5	0.0033	4
3	Injection	0.0041	1	0.0017	1	0.0010	1	0.0007	1
4	Inter-injection	0.0513	5	0.0402	5	0.0257	6	0.0023	3
5	Injection	0.0161	2	0.0208	4	0.0095	2	0.00490	6
6	Post-injection	0.1043	6	0.0941	6	0.0160	3	0.0011	2

* Sums of the squares of the deviations of the pounds of fat of the 10 non-transitional milkings from the corresponding averages were used to indicate the variability in pounds of fat.

Table 9 gives for total fat the values of the measure of within-day variability (excluding the two transitional milkings) adopted in connection with per cent fat and total milk. In the case of total fat, however, the tendency for the variability to be below average on injection days and high on post-injection days is somewhat better defined than in the case of per cent fat, where no common feature presents itself.

DISCUSSION

Milking cows normally at 2-hour intervals resulted in extreme milking-to-milking variability in production. These results indicate that the cows were incapable of responding at 2-hour intervals to the normal stimulus of milking by a "let-down" of milk. The milking-to-milking variability of production was greatly reduced when oxytocin was used to facilitate a "let-down".

Conclusions drawn by Ragsdale *et al.* (8), Garrison and Turner (4), and Gasnier *et al.* (5) may be in error if a "let-down" cannot be elicited at 0.5-1-, and 2-hour intervals, as multiples of production obtained at those intervals would not give a true trend of milk secretion over longer periods. The first milking on an injection day, with one exception, exceeded the maximum production of any of the other milkings of the same day, which indicates that all the milk being produced is not removed when cows are milked at short intervals without the use of oxytocin.

Turner (11) postulates that the pressure due to the accumulation of milk within the lumina of the alveoli becomes sufficiently great to prevent the normal excretion of milk by rupture of the cell membrane. At high pressures, only those constituents of milk which are permeable to the cell membranes pass out of the cells into the lumen of the alveolus. The fat globules which are in suspension are retained in the cells. According to Turner, this theory explains the decrease in the per cent of fat concomitant to a lengthened milking interval, and the increase in per cent of fat in advanced lactation and milk strippings. If this theory is correct, the milk produced at short milking intervals at a low alveolar pressure should contain a higher per cent of fat than milk produced at longer milking intervals. In our experiments the per cent of fat, with one exception, was least during the 12-time-a-day milking period, when according to Turner's theory the per cent of fat in the milk should have been highest. However, the daily fat production was greatest with two exceptions during the 12-time-a-day period as a result of an increased flow of milk and not an increase in per cent of fat.

The data indicate that milk secretion proceeds at a uniform rate when cows are milked at 2-hour intervals, which verifies the results obtained by Shaw (9). However, at variance with Shaw's data was an increase in total milk and fat production by a more frequent removal of milk.

SUMMARY

A complete removal of accumulated milk was not obtained at each 2-hour milking interval when cows were milked normally, resulting in variable production from milking to milking.

Injections of 10 I.U. of oxytocin just prior to each 2-hour milking interval greatly decreased the milking-to-milking variability of production.

When cows were milked 12 times a day, total fat production, with two exceptions, was greater than in the pre- and post-experimental periods when the cows were milked twice daily. The yield of non-fat milk constituents showed an even greater increase, resulting in a lowered percentage of fat,. with one exception, during the experimental period.

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THE AMOUNT OF DDT FOUND IN THE MILK OF COWS FOLLOWING SPRAYING[1]

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When it was shown that DDT may be absorbed through the skin of animals (2, 5) and that animals fed massive doses of this material produced milk containing toxic doses of DDT (1, 6, 7), considerable interest was created in the possibility that cows sprayed with this insecticide for fly control might produce milk containing toxic amounts of DDT. To test this hypothesis a cooperative experiment was planned to show the effects of very heavy spraying and also the effects of the spraying schedule suggested for hornfly control in this area.

Sixteen cows were selected from the Experiment Station Dairy Herd in such a way that no two experimental cows stood side by side. They were divided into four lots, each containing two Holsteins and two Jerseys. Except for the spraying, experimental cows were handled in the same manner as the regular milking herd. No precautions were taken to prevent the animals from obtaining DDT by licking themselves or other test animals. The test animals were not segregated from the rest of the herd and were pastured, except during milking hours. Milk used as the control was secured from cows stanchioned in another section of the dairy barn. The udders of all cows were thoroughly washed before milking with a solution containing 200 p.p.m. available chlorine. The entire herd had been sprayed with 0.25 per cent DDT 2.5 months preceding the initial experimental spraying.

Lot I was sprayed daily with 2 quarts of 5 per cent water suspensible DDT spray per animal and lot II with 2 quarts of 1 per cent emulsion. (This latter lot was sprayed with 5 per cent emulsion for the first 4 days but it became necessary to decrease the concentration to avoid further "burning" by the solvent.) Lots III and IV were sprayed on the same day every other week with 2 quarts of 0.25 per cent DDT spray. Material for lot III was made of water suspensible powder and that for lot IV of an emulsion. All spraying was done with a 3-gallon compressed-air sprayer after the evening milking. An attempt was made to cover the entire animal each time. Spraying was started July 15 and discontinued August 30.

Milk samples were taken in the evening directly from the milking machine and were transferred to the Department of Agricultural Chemistry Research, where they were analyzed for DDT content. The samples of milk

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were stored in a refrigerator and the DDT separated from the milk and concentrated by a slight modification of the method of Schechter *et al.* (3). The color was developed by the procedure set forth by Schechter *et al.* (4). Color densities were determined in the earlier samples by using the Bausch and Lomb colorimeter. The later determinations were made with the Coleman spectrophotometer at 596 mμ. In every case milk free from DDT and milk containing known amounts of DDT were run as controls. Samples were taken at weekly intervals.

To obtain a more comprehensive picture of the elimination of DDT through the milk, a single animal, no. 17, was sprayed and sampled daily. The spray used was composed of 2 quarts of 5 per cent water suspensible DDT. This animal was sprayed first on August 10 and sprayed last on August 30.

TABLE 1

Amounts of DDT appearing in the milk of cows sprayed with DDT

No. of cow	Breed of cow	Type of DDT	Conc. DDT (%)	Interval between spraying[a]	Max. amt. DDT in milk in p.p.m.	Duration of DDT elimination in milk[b]
1	Jersey	Wettable powd.	5.0	Daily	20	126[f]
2	Holstein	Wettable powd.	5.0	Daily	18	21
3	Jersey	Wettable powd.	5.0	Daily	+	
4	Holstein	Wettable powd.	5.0	Daily	+	
5	Jersey	Emulsion	1.0[d]	Daily	19	56
6	Holstein	Emulsion	1.0[d]	Daily	19	56
7	Jersey	Emulsion	1.0[d]	Daily	+	
8	Holstein	Emulsion	1.0[d]	Daily	+	
9	Jersey	Wettable powd.	0.25	14 days	1.5	21
10	Holstein	Wettable powd.	0.25	14 days	Trace	7
11	Jersey	Wettable powd.	0.25	14 days	+	
12	Holstein	Wettable powd.	0.25	14 days	+	
13	Jersey	Emulsion	0.25	14 days	2.5	21
14	Holstein	Emulsion	0.25	14 days	0.20	7
15	Jersey	Emulsion	0.25	14 days	+	
16	Holstein	Emulsion	0.25	14 days	+	
17	Jersey	Wettable powd.[c]	5.0	Daily[e]	33.6	119[f]

[a] Sprayed from July 15 to August 30.

[b] The time in days elapsing between the initial positive analysis and the first negative one.

[c] Milk from this animal was analyzed daily for DDT.

[d] During the first 4 days these animals were sprayed with 5% DDT but it then was necessary to drop the concentration to 1% to avoid further injury.

[e] Sprayed from August 10 to August 30.

[f] End of lactation period.

RESULTS

Milk samples collected from experimental cows 3 and 10 days after spraying were entirely negative for DDT. All samples taken the seventeenth day after spraying was started were positive to qualitative tests, but no quantitative tests were run.[2] The following week quantitative tests indicated that animals sprayed with 1 per cent emulsion were giving off 18 to 19 p.p.m. of DDT in the milk, while those sprayed with 5 per cent wettable powder produced milk containing 5–12 p.p.m. Animals sprayed with 0.25 per cent showed less than 1 p.p.m. The maximum concentration of DDT, 33.6 p.p.m., appeared in the milk 20 days after spraying started. At the end of their lactation period, 119 and 126 days after the final spraying, two cows still were giving off appreciable quantities of DDT. Samples of milk collected from one cow immediately after calving and 24, 48, 72, and 168 hours later were negative for DDT.[3] Under the conditions of the experiment, the milk produced by Jerseys generally contained more DDT than that of the Holsteins. The details of this experiment are shown in table 1.

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[2] To reduce the volume of chemical work, milk was analyzed from only one Holstein and one Jersey of each lot of cows after it was shown that the milk of all experimental animals contained DDT.

TABLE 2

Parts per million of DDT appearing in milk of cow sprayed daily with 2 quarts of 5 per cent DDT

Date	Aug.	Sept.	Oct.	Nov.	Dec.
1		24.2	4.0	3.4	Trace
2		16.9	3.7	3.9	2.1
3		11.9	2.9		2.7
4		14.9	2.8	4.6	2.8
5		12.6	2.6	4.0	2.1
6		11.2	3.8	4.2	2.9
7		8.7	3.1	3.8	2.9
8		9.4	2.4	3.1	2.8
9		7.5	3.3	3.4	2.8
10		7.0	1.8	4.8	2.8
11		6.0	3.3	4.2	3.0
12		8.1	2.1	5.0	2.6
13		3.2	1.9	3.0	
14		7.5	1.7	4.6	
15			2.3	3.6	
16	2.0	5.4	2.5	3.1	1.8
17	3.0	7.8	1.3	3.9	
18	2.9	4.6	1.8	2.0	
19	2.9	4.2	1.2	1.2	1.3
20	10.1	2.1	1.1	2.0	
21	8.5	2.9	1.1	3.0	
22	15.0	2.8	1.4	3.2	
23	6.3	2.2	1.7	2.7	
24	8.1	3.1	1.4	2.6	
25	11.6	2.9	2.0	3.8	
26	8.4	2.6	1.0	4.3	
27	8.0	3.4	2.5	4.1	
28	5.3	3.0	3.2	3.5	
29	18.5	4.4	2.7	2.7	
30	33.6[a]	3.5	2.2	1.8	
31	24.0		0.9		

[a] Spraying discontinued.

Table 2 shows the results of the detailed experiment involving a single cow sprayed and sampled daily. Six days after the initial spraying, 2 p.p.m. of DDT were found in the milk. Two weeks later 33.6 p.p.m. were found.

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[3] This animal calved 217 days after her last spraying.

While spraying was in progress, analyses varied widely, perhaps due to contamination. After spraying was stopped, results were more uniform. More DDT appeared in the milk during the third month after spraying was stopped than during the second month.

BIOLOGICAL TESTS

Milk from test cows showing the highest quantity of DDT was fed to 12 young mice. They were forced to consume as much as possible by mixing all food with the milk and supplying this milk as the only liquid to drink for a period of 4 months. No demonstrable differences were noted in the test mice or their progeny when compared with control mice fed in a similar manner using normal milk.

Third instar larvae of mosquitoes, *Aedes aegypti* (Linn.) and *Culex quinquifasciatus* Say, were placed in dilutions of milk from cow no. 17 when it was showing the largest amounts of DDT. Larvae in both check and test milk were unable to live unless the concentration of milk was reduced below 50 per cent. Below this point larvae developed normally in both test and check series, and no evidence of DDT toxicity was noted. In addition, known amounts of DDT in acetone were added to milk diluted to half strength with water. The mixture then was homogenized to decrease the smothering effect due to the cream. No toxicity to larvae was noted in mixtures containing below 20 p.p.m. while above 50 p.p.m., all larvae were killed. In water, less than 0.1 p.p.m. kills all mosquito larvae. Little difference was noted in the susceptibility of *Aedes aegypti* and *Culex quinquifasciatus.*

Milk from cow no. 17 was supplied to cages of flies and mortality determinations were made after 72 hours. The death rates in the check cages were slightly higher than in the control lot, but no typical symptoms of DDT poisoning were found. Flies fed milk to which 50 p.p.m. DDT had been added lived slightly longer than flies from the same lot fed untreated milk.

DISCUSSION

Since it was the purpose of these experiments to demonstrate the amount of DDT that might appear in the milk of cows as the result of spraying, no precautions were taken to prevent the cows from licking themselves or other test animals. The data accumulated suggest that very small amounts of DDT may appear in the milk even though minimum spray schedules are followed. If very heavy applications are used, as much as 33.6 p.p.m. may be found in the milk at times. However, biological tests with mice, mosquito larvae, and flies suggest that even maximum amounts do not cause appreciable toxicity. When large quantities are used, DDT apparently is stored in the cow's body and eliminated in the milk for extended periods.

Biological tests have failed to demonstrate toxicity in milk from cattle sprayed with DDT, even though they were treated with many times the amount that normally would be used for arthropod control. Excessive amounts of emulsion caused irritation, but no injury was noted in cattle sprayed with the same concentration of wettable powder DDT spray.

SUMMARY

Four lots of cattle were sprayed with DDT to determine the amount of this material that may appear in the milk following recommended and excessive treatment.

All animals tested excreted some DDT in the milk.

Animals sprayed with excessive amounts continued to eliminate DDT until the end of their lactation period, 119 and 126 days after the final spraying, but none after freshening.

Biological tests for toxicity of the milk to mice, mosquito larvae, and flies were negative.

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THE EFFICIENCY OF THE FARM-SIZE MECHANICAL SEPARATOR IN WITHHOLDING EXTRANEOUS MATTER FROM CREAM[1,2]

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The problem of extraneous matter in farm-separated cream is becoming increasingly important. In order to develop effective control measures, information is needed on the amount of contamination occurring at the various stages of production and marketing of cream. The possible sources of contamination of milk are fairly well established. The first stage of study on sources of sediment contained in cream when it arrives at the creamery would seem to be as the cream comes from the separator. The centrifugal action of the mechanical cream separator bowl together with the accumulation of separator slime during the separation process raised the question regarding the efficacy of the separator as a means of removing extraneous matter from cream during the separation process. The question also arises as to whether the mechanical cream separator will remove some types of material and not others. The amount of sediment found in cream as it comes from the separator will indicate whether primary emphasis should be placed on production practices prior to separation or on later stages of handling, if cream relatively free from sediment is to be obtained.

Boxell (1) has reported that cream obtained by mechanical separation on the farm had a lower sediment content on arrival at the creamery than did cream obtained by other methods. Experimental trials by the Research Committee of the American Butter Institute (5), in which split lots of the same milk were separated by mechanical separation, water dilution, and hand skimming, showed that the cream obtained by the different methods gave sediment tests scoring 1, 2, and 2 +, respectively, by the American Butter Institute cream sediment standard (1946). In spite of the long-time extensive use of mechanical cream separators on farms and the relatively long existence of the sediment problem in cream, no other published experimental data pertinent to the problem have been found.

Differences of opinion on the subject are indicated by the fact that a representative of one of the large manufacturers of cream separators (4) stated that the mechanical cream separator should not be expected to be an effective means of removing extraneous matter from cream. On the other hand, Judkins and Mack (3), in listing the advantages of the farm-size

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mechanical separator, state in their textbook that the cream is free from dirt because the separator acts as a clarifier. The objectives of the experiments herein reported were to determine the effectiveness of mechanical cream separation in preventing sediment in the milk from entering the cream under varying conditions and with different kinds of extraneous matter.

METHODS

In the laboratory trials two makes of farm-size, power-driven separators were used; one had a capacity of 800 lb. per hour and the other of 750 lb. per hour. The separators were washed, assembled, and flushed with warm water immediately prior to each use except in trials otherwise specified. Approximately 40 lb. of whole milk were used for most trials, since this amount was considered about average for Kansas cream-producing farms. Occasionally, larger quantities were separated as indicated. The milk was warmed to about 100° F., sampled for sediment testing, and separated. The milk sample was not taken in the customary off-the-bottom manner used on receiving platforms, but was obtained by stirring thoroughly and taking a pint sample. Where considerable farm contamination was present, no additional material was added to the milk; but when the milk was relatively clean, various amounts of dust, soil, manure, and combinations thereof were added prior to separation. One quart of sediment-free water was used to flush the separator bowl after separation was completed, by pouring the water into the supply tank. In some of the trials the flush water from the cream spout was collected separately, not being allowed to enter the cream. Slightly over 4 pints of cream and about one-half pint of cream flush were obtained from each separation. After separation, the separator parts were examined to determine where the extraneous material was lodged. With a few exceptions, sediment tests were made on 2 ounces, 1 pint, and 3 pints of the mixed cream, and on the cream flush when the latter was collected separately. The 2-ounce quantity was used since it is the one commonly tested in cream stations and is the amount upon which the American Butter Institute cream sediment standards (1946) are based. The 1-pint quantity was used because it is the amount often employed in plant tests and regulatory examinations. The 3-pint quantity represented most of the remaining cream from 40 lb. of milk and permitted a more critical evaluation of the amount of sediment in the cream. Standard 1¼″ lintine sediment test discs were used with a laboratory suction filtering device. Since the cream was sweet, only warm water was required to assist filtering. Sediment pads were examined both with and without the wide field microscope and scored according to the following modification of the American Butter Institute cream sediment standards, using plus or minus signs to indicate variations.

American Butter Institute cream sediment score:	1	2	3
Corresponding scores used in this study	1, 2, 3	4, 5, 6	7, 8, 9

To determine the situation under field conditions, samples were obtained at five cream-producing farms. In these farm trials the separator was prepared and operated by the producer. The milk was sampled from the separator supply tank into which it usually was strained. Two-ounce and 1-pint samples of the mixed cream were obtained for sediment testing. The flushing was done by the producer in his usual manner. Both milk and cream samples were sediment tested on the farm with one of the common types of cream sediment testers, warmed filtered water from the laboratory being used on the cream. Generally, the water used for flushing also was tested for sediment. Information was obtained relative to the age and general condition of the separator and the amount of milk sepa-

TABLE 1

Efficiency of the farm-size mechanical separator in withholding extraneous matter from cream

(Laboratory trials with the cream flush collected separately)

Trial	Lb. milk used	Material added to milk	Sediment score				
			Whole milk	Cream			Cream flush
			1 pint	2 oz.	1 pint	3 pints	½ pint
1	40	None	8			5	4
2	40	None	5	1	1	3	3
3	250	None	9	1	1		
4	350	None	9		3		3
5	600	None	4	1	1	2	2
6	40	0.1 g. road dust	7		4	5	2
7	40	0.1 g. road dust	7	1	1	3	4
8	40	0.15 g. dry manure	14*		5	11*	8
9	40	0.05 g. dry manure	9			5	6
10	40	0.2 g. fresh manure	8	1	2	5	3
11†	40	0.2 g. fresh manure	8	1	2	5	5
12‡	40	0.2 g. fresh manure	8	2	5	5	5
13	40	0.1 g. soil	9	1	2	3	5
14†	40	0.1 g. soil	11*	3	3	5	7
15‡	40	0.1 g. soil	12*	3	5	7	7
Approx. av. score ...			9 −	1 +	3 −	5	5 −
Approx. A.B.I. score§ ...			3 +	1 −	1	2	2

* Score approximated.

† Separator unwashed from previous trial (1 day).

‡ Separator unwashed from two previous trials (2 days).

§ American Butter Institute cream sediment standard (1946).

rated. The sediment pads were examined and scored by the method previously mentioned.

RESULTS

Results of laboratory trials in using mechanical separation as a means of withholding extraneous matter from cream when various amounts and types of material had been added to the milk are shown in table 1. In the trials involved, the flush water from the cream spout was collected and tested separately. Milk with high sediment scores purposely was used to evaluate under extreme conditions the efficiency of the mechanical separation process in withholding extraneous matter. Thirteen lots had a sediment score equivalent to 3 or more by the American Butter Institute standard and would have been reject milk on the basis of sediment. The approximate average score was 9 – by the modified score used in this study and 3 + by the A.B.I. standard.

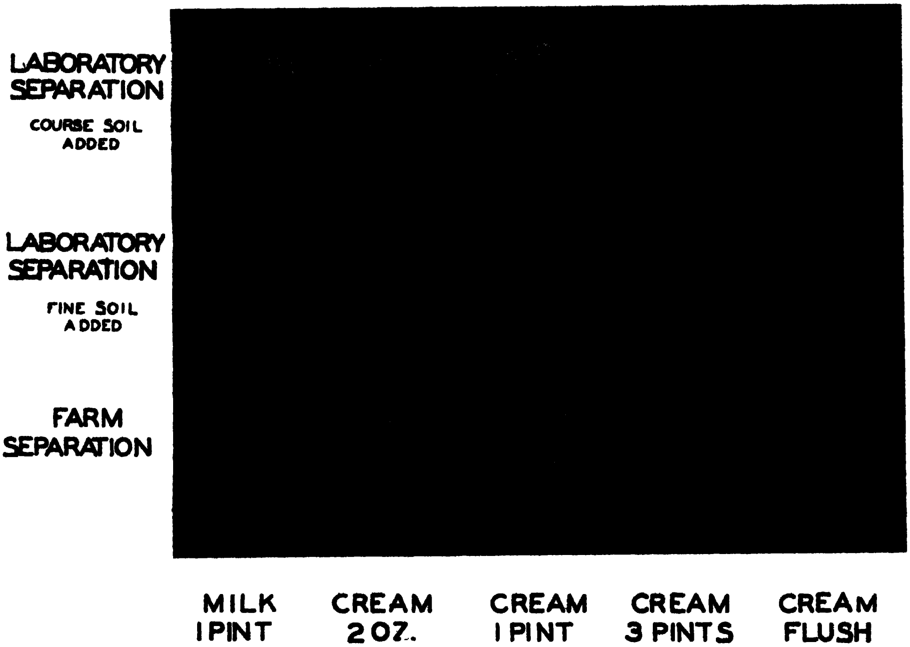

FIG. 1. Sediment tests of milk, cream, and cream flush from representative separation trials.

When sediment tests were made on the cream, the amount obtained from a 2-ounce sample was very small and usually was evident only on close macroscopic inspection, the average score being 1 + by the system used in this study and 1 – by the A.B.I. standard. As would be expected, the larger samples had a higher sediment score, the 1-pint samples averaging 3 – (A.B.I. score 1) and the 3-pint samples averaging 5 (A.B.I. score 2).

Considering the high sediment content of the milk used in these laboratory trials, with the flush water withheld from the cream, the results would seem to justify the conclusion that the mechanical separator was relatively effective in withholding from cream most of the sediment present in the milk. It resulted in cream of acceptable sediment score by prevailing standards. Representative results are shown in figure 1.

The 15 lots of milk involved in these laboratory trials included five lots to which no additional extraneous matter was added, two lots to which road dust was added, two lots to which dry manure was added, three lots to which fresh manure was added, and three lots to which light soil was added. Although comparison among trials using the same type of material was possible, it was difficult to make comparisons between trials with different types of sediment because of the difference in amounts used and the variation in size and number of particles involved. In general, however, when the added material contained a high proportion of large-sized, light weight particles (chaff, dried manure, and leaf fragments), more sediment escaped into the cream, while the heavier particles (sand, clay, and coal dust) were withheld.

As separator slime accumulates in the bowl, the separator conceivably might become less effective in withholding extraneous matter from the cream. To investigate this factor, three larger lots of milk (250, 350, and 600 lb. in trials 3, 4, and 5, respectively) with considerable farm contamination were separated and cream samples were obtained toward the end of the separation process. The sediment scores on the cream samples thus obtained were not significantly different from those in other trials where samples were taken from thoroughly stirred, entire lots of cream obtained from the separation of 40-lb. quantities of milk. The large quantities of high sediment-content milk used, which would represent the extreme in volume that might be expected under farm conditions, were not sufficient to cause enough accumulation of sludge in the bowl to impair the separator efficiency in removing extraneous matter. While cow hairs usually were present in the farm-contaminated milk, they rarely were found in the cream or cream flush samples tested for sediment. Hairs were found in the bowl sludge chamber; in instances where the hair contamination in the milk had been heavy, some were found near the cream ports in the lower discs.

To determine whether failure to wash the separator affects its efficiency in removing extraneous material, trials were conducted in which the same separator was used both clean and unwashed. Equal amounts of milk with specific amounts of added contaminating material were used (trials 10 to 15). After using the clean separator it was left unwashed one day before the next trial, and then left unwashed an additional day before using it again. Under the conditions of these trials the increases in the sediment scores of the cream samples were not as marked as might be expected. It was noted that a slight amount of rust was expelled into the cream after the separator had remained unwashed and assembled. Rusted spots were evident in the machine when it was dismantled.

As previously mentioned, in the above trials the flush water expelled through the cream spout was kept separate from the cream. The sediment score of this cream flush was related to the sediment score of the original milk and to a lesser extent to the sediment score of the cream. The average score of the flush was 5 – (A.B.I. score 2) which was about the same score as on 3 pints of cream, although the cream flush involved only about one-half pint. Thus, on the basis of comparative volume, the cream flush contained about six times as much sediment as the cream.

TABLE 2

Efficiency of the farm-size mechanical separator in withholding extraneous matter from cream

(Laboratory trials using 40 lb. milk with the cream flush collected in the cream)

Trial*	Sediment score			
	Whole milk	Cream		
	1 pint	2 oz.	1 pint	3 pints
1	7	1	2	4
2	6	1	1	4
3	6	1	2	5
4	7	1	2	3
5	6	1	2	3
Approx. av.	6 +	1	2	4 –
Approx. A.B.I. score	2 +	1 –	1	2 –

* In trials 1, 2, and 3, 0.15 g. of soil-manure mixture was added to each lot of milk. In trials 4 and 5 the milk contained only the original contamination.

In five trials (table 2) in which the cream flush was allowed to enter the cream, the average sediment score of 2-ounce samples was 1 (A.B.I. score 1 –), of 1-pint samples it was 2 (A.B.I. score 1), and of 3 pints it was 4 – (A.B.I. score 2 –). These results compare favorably with those reported in table 1, in which the cream flush was kept separate from the cream, but the sediment score of the milk used was uniformly better. Although the cream flush apparently was not of sufficient volume to change materially the sediment score of the cream, the excessively high sediment content of the cream flush as shown in table 1 would indicate that withholding it from the cream would be of definite advantage in keeping sediment out of cream.

Since observations in previous trials had indicated that the separator removed most of the cow hairs present in the milk, seven laboratory trials were conducted to determine the efficiency of the separator in withholding rodent hairs added to the milk, because of the possibility of this type of contamination on the farm (2) (table 3). The degree of contamination of the milk and the cream was measured by counting the number of rodent hairs found on the sediment pads. The results showed that the separator definitely was effective in withholding rodent hairs from the cream, but an occasional hair was found in the larger samples, indicating that some may escape into the cream. Assuming the number of hairs found in the pint samples of milk to be representative, there would be an average of about 360 hairs in the 40-lb. lots of milk separated. Since practically all the cream from each lot of milk was used in the three different sediment tests on the cream, it may be concluded that the separator removed better than 95 per cent of the hairs. In the three trials where the cream flush was kept separate from the cream, the number of hairs in the flush was not markedly different from that in the cream. Also, the number of hairs found in cream which included the cream flush was little different from that in the cream where the flush was held separately. Examination of the interior of the separator bowl showed the hairs to be entrapped in the sludge chamber. These results would indicate that rodent hairs found in farm-separated cream would consist primarily of contamination after separation.

TABLE 3

Efficiency of the farm-size mechanical separator in withholding rodent hairs from cream
(Laboratory trials with rodent hairs added to 40 lb. of milk before separating)

Trial	No. of rodent hairs on sediment pads				
	Whole milk	Cream			Cream flush
	1 pint	2 oz.	1 pint	3 pints	¼ pint
	Cream flush collected separately				
1	22			1	0
2	6	0	0	0	1
3	8	0	0	2	1
Av.	12	0	0	1	< 1
	Cream flush collected in cream				
4	6	0	1	1	
5	11	0	0	1	
6	4	0	0	1	
7	5	0	0	1	
Av.	6	0	< 1	1	

The preceding studies of the efficiency of the cream separator in removing various types of extraneous matter were conducted under more ideal conditions than generally would be found on the farm. Therefore, eight farm trials were made as a check against the laboratory findings. The separators were of smaller capacity than those used in the laboratory, and ranged from 4 to 8 years of age. In each instance they had been washed since previous use. The milk separated generally was cleaner than that selected for use in the laboratory.

In the farm trials the separator again had a considerable clarifying effect on the cream (table 4). In one trial, rodent-type hairs were found in the milk but none was found in the cream samples tested. However, the removal of sediment in the farm trials was not so complete as in the laboratory trials. Considering the better quality of milk involved, this suggests the need for careful attention to separating conditions to obtain best results. The cream flush contained considerably more sediment than the cream portions, considering the volume involved, but generally less than the cream flush in the laboratory trials. The influence of collecting the cream flush in the cream was most marked in the pint samples, resulting in about twice as much sediment as when the flush was withheld. The sediment score on the water used for flushing was higher than on the cream and about the same as on the original milk. This may have had an influence on the increased sediment content of the pint samples when the cream flush was included. Nevertheless, under farm conditions the cream from the separator was reasonably

TABLE 4

Efficiency of the farm-size mechanical separator in withholding extraneous matter from cream

(Farm trials)

Trial	Approx. lb. milk	Sediment score				
		Whole milk	Cream		Cream flush	Flush water
		1 pint	2 oz.	1 pint	½ pint	1 pint
Cream flush collected separately						
1	40	8	4	4	5	
2	15	2	1	1	3	5
3	40	5	1	2	4	6
4	70	6	2	2	3	
Approx. av.	40	5 +	2	2 +	4 −	5 +
Approx. A.B.I. score		2	1	1	2	2
Cream flush collected in cream						
5	45	6	2	5		7
6	40	6	3	4		3
7	30	1	2	3		6
8	40	4	2	4		4
Approx. av.	40	4 +	2 +	4		5
Approx. A.B.I. score		2 −	1	2		2

clean as measured by the sediment test on the commonly used 2-ounce size cream samples.

CONCLUSIONS

1. Under laboratory conditions and using high sediment content milk, cream from farm-size mechanical separators was practically clean as judged by present widely used methods of testing, having a no. 1 sediment score or better (A.B.I. standard). Even under farm conditions where no special precautions were taken, reasonably clean cream was obtained with no. 1 sediment scores on 2-ounce samples.

2. In general, when the extraneous matter in the milk contained a high proportion of large-sized, light weight particles, more sediment escaped into the cream, while the heavier particles were withheld. Cow hairs rarely were found in the cream.

3. The separation of large quantities of milk, which would represent the extreme in volume that might be expected under farm conditions, did not impair the separator efficiency in removing extraneous matter, even though the milk used had a high sediment content.

4. In the laboratory, failure to wash the separator did not result in as marked an increase in the sediment content of the cream as might be expected.

5. The farm-size mechanical separator was very effective in withholding rodent hairs from cream.

6. The cream flush contained proportionately more sediment than the cream, which would suggest that withholding it from the cream would be a possible measure in reducing sediment.

7. In the practical control of sediment in cream where mechanical separators are used, it would appear that, along with reasonable precautions in the separation process, more emphasis should be placed on attention during later stages of handling.

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FROZEN HOMOGENIZED MILK. III. STABILITY OF MILK SOLIDS DISTRIBUTION IN FROZEN HOMOGENIZED MILK

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Previous studies (1, 3, 4, 5, 7) have shown that freezing and storage temperature affect the physical character of homogenized milk. Later studies (2, 6) showed that when homogenized milk is frozen, the solid components tend to concentrate in the lower portions of the sample. Changes in the temperature at which frozen homogenized milk was stored did not, however, materially affect the chemical composition of the different sections of quart samples.

The results reported by the authors (2) were based in part on samples of homogenized milk frozen and stored under conditions simulating those encountered in transferring milk from land storage to storage on hospital ships. The results obtained on the chemical analysis of some of the individual samples indicated that the chemical constituents may continue to shift after the milk is frozen. Since the literature does not contain information regarding the movement of the chemical constituents in frozen homogenized milk, the present study was undertaken to determine whether or not the solid constituents do continue to shift after the milk is frozen.

PROCEDURE

Four groups of homogenized milk samples with a fat content of 3.8 per cent and packaged in quart paper containers by a commercial dairy in Washington, D. C., were frozen at – 11.5° C., – 17.8° C., – 27.5° C., and – 36° C. and held at these respective temperatures until they were removed for chemical examinations. The temperature of storage remained constant for all groups except one. Within 3 days after the milk was placed in storage at – 17.8° C., the temperature rose to – 16.7° C., and for the next 10 days it varied from – 16.7 to – 15.0° C. Furthermore, during the entire storage period there were times when the temperature rose as high as – 12.2° C. To insure that initial freezing had taken place, a sample of each group was removed at the end of the third day. Subsequent samples were removed at 10-day intervals. All samples were divided, while frozen, into two equal halves and allowed to thaw at room temperature. Both the top and bottom sections were analyzed for protein, fat, total solids, lactose, and chlorides by

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TABLE 1

Chemical analyses of different sections of homogenized milk frozen and held at constant temperatures

Storage time (days)	% protein (N × 6.38)		% fat		% total solids		% lactose		% chlorides	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
A. Frozen and held at – 11.5° C.										
3*	2.74	3.91	2.85	3.94	10.06	14.03	3.94	5.38	0.084	0.116
13	2.54	4.17	2.64	4.15	9.21	14.94	3.56	5.71	0.077	0.123
25	2.98	4.22	2.80	4.24	9.28	15.10	3.54	5.86	0.076	0.124
34	2.69	3.97	2.87	4.04	9.95	14.38	4.01	5.67	0.083	0.118
43	2.66	3.92	2.78	3.85	9.68	14.04	3.84	5.44	0.090	0.115
54	2.45	4.22	2.59	4.22	8.78	15.04	3.33	5.78	0.073	0.124
64	2.50	4.25	2.61	4.19	8.95	14.98	3.53	5.82	0.072	0.121
74	2.49	4.25	2.56	4.18	8.78	14.95	3.41	5.72	0.070	0.122
84	2.50	4.30	2.55	4.10	8.89	15.11	3.46	5.93	0.074	0.123
96	2.46	4.25	2.55	4.15	8.79	15.01	3.59	5.99	0.072	0.123
104	2.43	4.17	2.54	4.02	8.88	14.83	3.55	5.86	0.072	0.120
113	2.57		2.62	3.95	9.36	14.66	3.64	5.63	0.075	0.118
B. Frozen and held at – 17.8° C.										
3*	2.75	3.96	3.00	4.21	10.01	14.41	3.83	5.61	0.077	0.114
13	2.81	3.89	3.04	4.15	10.29	14.30	3.92	5.51	0.081	0.112
23	2.75	3.99	2.98	4.20	10.02	14.68	3.78	5.65	0.078	0.115
34	2.73	3.95	2.98	4.22	10.09	14.61	3.77	5.61	0.079	0.114
43	2.68	4.00	2.91	4.35	9.80	14.74	3.75	5.66	0.078	0.116
52	2.79	3.94	3.04	4.17	10.18	14.36	3.83	5.52	0.081	0.114
63	2.83	3.93	2.99	4.15	10.29	14.43	3.90	5.51	0.081	0.112
73	2.78	3.96	3.03	4.17	10.19	14.42	3.94	5.61	0.081	0.113
83	2.66	4.07	2.88	4.23	9.73	14.65	3.70	5.79	0.077	0.115
93	2.69	4.04	2.92	4.20	9.82	14.81	3.83	5.75	0.075	0.115
103	2.78	3.63	2.87	3.72	9.93	15.07	3.79	5.88	0.077	0.118
C. Frozen and held at – 27.5° C.										
3*	2.88	3.98	2.96	4.01	10.23	14.47	3.96	5.73	0.082	0.112
13	2.76	4.04	2.88	4.08	9.89	14.70	3.67	5.84	0.077	0.116
25	2.57	3.88	3.14	3.96	10.93	13.99	4.26	5.37	0.097	0.112
34	2.98	3.74	3.13	3.88	10.91	13.69	4.23	5.40	0.092	0.112
43	2.85	3.94	2.94	4.00	10.08	14.52	3.85	5.84	0.079	0.126
54	2.97	3.89	3.04	4.03	10.74	13.87	4.14	5.31	0.090	0.113
64	3.05	3.86	3.15	3.87	10.64	13.90	4.07	5.45	0.082	0.119
74	3.01	3.87	3.06	3.88	10.68	13.70	4.08	5.35	0.089	0.112
84	3.05	4.04	3.09	3.91	10.87	13.74	4.27	5.32	0.091	0.112
96	2.99	3.88	3.08	3.91	10.63	13.88	4.02	5.30	0.089	0.113
104	3.04	3.85	3.08	3.86	10.87	13.65	4.35	5.37	0.092	0.112
113	2.96	3.86	3.08	3.92	10.38	14.03	3.79	5.47	0.081	0.120
123	2.85	4.00	2.93	4.00	10.01	14.41	3.88	5.90	0.081	0.121
133	3.01	3.86	3.07	3.92	10.53	13.71	3.96	5.40	0.087	0.111
144	2.98	3.83	3.11	3.89	10.51	13.89	4.05	5.60	0.084	0.120
154	2.74	3.98	2.89	4.06	9.83	14.48	3.78	5.97	0.078	0.124
D. Frozen and held at – 36° C.										
3*	2.97	3.94	3.21	4.20	10.84	14.35	4.07	5.46	0.086	0.108
13	2.88	3.92	3.16	4.19	10.80	14.34	4.06	5.50	0.085	0.112
24	2.91	3.88	3.19	4.17	10.73	14.37	4.11	5.56	0.085	0.112
33	2.87	3.89	3.11	4.16	10.54	14.32	4.07	5.49	0.085	0.112
42	2.94	3.86	3.16	4.01	10.83	14.21	4.14	5.40	0.086	0.110
53	2.93	3.88	3.13	4.12	10.75	14.24	4.08	5.42	0.086	0.111
63	2.78	3.97	3.02	4.24	10.35	14.68	4.09	5.68	0.085	0.116
73†	2.93	3.92	3.17	4.19	10.67	14.40	3.99	5.41	0.087	0.113

* Initial.

† Mechanical failure of deep freezer occurred shortly after the 73rd day.

methods described in a previous report (2). After the samples had thawed, they were preserved by adding 1 ml. of formalin.

EXPERIMENTAL RESULTS

When homogenized milk was frozen and held at constant temperatures, *i.e.*, –11.5, –27.5, and –36.0° C., and at approximately –17.8° C., the concentration of milk solids was greater in the bottom section of the quart container than it was in the top section. The quantity of milk solids found in the top section ranged from 2.43 to 3.05 per cent protein, 2.54 to 3.21 per cent fat, 8.78 to 10.93 per cent total solids, 3.33 to 4.35 per cent lactose, and 0.070 to 0.097 per cent chlorides. In the bottom section the milk solids ranged from 3.63 to 4.30 per cent protein, 3.72 to 4.35 per cent fat, 13.65 to 15.11 per cent total solids, 5.30 to 5.99 per cent lactose, and 0.108 to 0.126 per cent chlorides.

After the milk was initially frozen, the quantity of each of the solid constituents found in the top and bottom sections remained practically constant (table 1). When the milk was frozen and held at –11.5° C., the greatest differences between the initial analysis and any subsequent analysis of the top and bottom sections, respectively, were as follows: protein, 0.31 and 0.39; fat, 0.31 and 0.30; total solids, 1.28 and 1.08; lactose, 0.61 and 0.61; and chlorides, 0.014 and 0.008. When the milk was frozen and held at approximately –17.8° C., the greatest differences were: protein, 0.09 and 0.33; fat, 0.13 and 0.49; total solids, 0.28 and 0.66; lactose, 0.13 and 0.27; and chlorides 0.004 and 0.004 for the top and bottom sections respectively. When the milk was frozen and held at –27.5° C., these differences were: protein, 0.31 and 0.24; fat, 0.19 and 0.15; total solids, 0.70 and 0.82; lactose, 0.39 and 0.43; and chlorides, 0.015 and 0.014. With milk frozen and held at –36.0° C., the differences in the solid constituents were: protein, 0.19 and 0.08; fat, 0.19 and 0.19; total solids, 0.49 and 0.33; lactose, 0.08 and 0.22; and chlorides, 0.001 and 0.008.

These differences from the initial analyses did not progressively increase or decrease with an increase in the storage time. This shows that the milk solids did not continue to shift after the initial freezing of the milk and that these slight differences may be attributed to experimental error in dividing the frozen sample into top and bottom sections or in some instances to the fact that the milk had separated to such an extent that it was impossible to obtain a representative sample.

It further can be determined from table 1 that after the milk was frozen initially, the quantity of each of the solid constituents found in the top and bottom sections was in close agreement with the average analysis (based on analyses made after different storage periods). The difference between the average and the initial analyses ranged from 0 to 0.16 and from 0.02 to 0.24 for the protein, from 0.03 to 0.19 and from 0.04 to 0.15 for the fat, from

0.02 to 0.84 and from 0.01 to 0.73 for the total solids, from 0.01 to 0.32 and from 0.03 to 0.35 for the lactose, and from 0 to 0.007 and from 0 to 0.005 for the chlorides in the top and bottom sections, respectively. This close agreement also shows that the milk solids did not continue to shift after the initial freezing of the milk.

CONCLUSION

When homogenized milk was frozen, the concentration of the milk solids in the lower portion of the sample took place during the freezing process and apparently there was no further movement of these solids after the milk was frozen.

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THE UTILIZATION OF THE MINERAL-ION EXCHANGE PRINCIPLE IN STABILIZING EVAPORATED MILK

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Prior to the classic works of Sommer and Hart (4, 5, 6) the problem of heat coagulation in evaporated milk was little understood. These workers demonstrated the importance of salt relationships in the mechanism of the heat stability of this product and, supported by extensive investigations, presented the well-known "salt balance" theory of protein stability. This theory was based on an assumption that a delicate balance exists between calcium and magnesium ions, on the one hand, and citrates and phosphates on the other. An unbalanced system, one way or the other, would cause coagulation during sterilization. These investigators further demonstrated the importance of an optimum calcium-casein balance and suggested that the rest of the calcium was associated with citrates and phosphates. Their work, as well as commercial practice in recent years, has demonstrated that instability in evaporated milk almost invariably is due to an excess of calcium and magnesium.

The use of stabilizing salts has been a standard practice in evaporated milk manufacture for many years. Many investigators have studied the stabilizing effects of many salts, particularly sodium citrate and disodium phosphate (1, 5, 6, 7). For the most part, the industry today employs disodium phosphate to control the heat stability of evaporated milk, a practice which has been necessary for the successful year-around manufacture of the product.

In 1933 Lyman *et al.* (3) reported that salt readjustments could be made in milk by treating the product with suitable base exchange silicates. Later, Lyman (2) was issued a patent on the use of this process in producing soft curd milk. The method called for the removal of a part of the calcium and phosphorus from milk by means of a base exchange process which consisted of a filter bed of synthetic zeolite sand acting in the sodium cycle.

Since the instability of evaporated milk during sterilization is due primarily to an excess of calcium and magnesium ions, it appeared logical that some system employing the ionic exchange principle might be applied to

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the problem of stabilizing this product. Such an investigation was undertaken and carried out over a period of 1 year.

EXPERIMENTAL PROCEDURE

Since excess calcium and magnesium ions primarily are responsible for the instability of milk proteins during the sterilization of evaporated milk, the need for a cationic type of exchanger with a high degree of specificity for polyvalent cations is obvious. The exchanger used in the investigation was a resinous type known as Amberlite IR—100. Preliminary trials demonstrated that other cation exchangers, such as the carbonaceous type, might have been used with equivalent success. When acting in the sodium cycle, these exchangers effect an efficient removal of calcium and magnesium from milk without materially affecting the anion concentration.

In processing the milk by the above mineral-ion exchange process, hereafter called the M.I.E. process, several variations were investigated. Both raw whole milks and skim milks were treated and employed in the evaporated milk experiments. Mechanical and technical difficulties resulting in the loss of butterfat in the filter beds made the treatment of skim milk the more effective and practical procedure. After several exploratory trials, the advantage of using a dried M.I.E.-treated skim milk was demonstrated, and most of the later trials were conducted with this material.

In passing milk through the cation exchanger, about 60 per cent of the calcium was removed. When dried by the spray process, the resulting powder was white, fluffy, and very soluble in water.

In this particular exchange process the pH of the milk was increased to approximately 8.0. In some cases the pH was adjusted back to 6.6 by means of a hydrogen exchanger so that a comparison of the effectiveness of these products could be made in evaporated milk.

The M.I.E.-treated milks were employed in (a) fluid, (b) concentrated (26.2 per cent total solids), and (c) powdered forms, and were added either to the fluid untreated milk before forewarming or to the concentrated milk prior to filling and sterilizing. All evaporated milks were standardized to 26.2 per cent total solids with fat-to-total solids ratio of 1 to 3.3.

Since the amount of "correction" of the salt equilibrium (removal of excess calcium) was very slight in most milks, only a small percentage of the fluid required treatment to produce optimum stability during sterilization. When the bulk lots of fluid or concentrated milk were treated with various percentages of M.I.E.-processed milk, this percentage was calculated and expressed as "per cent of treated milk added". In other words, a 1,000 lb. batch of fluid milk (or equivalent) testing 12.7 per cent total solids required the addition of 10 lb. of M.I.E.-treated fluid or about 1.3 lb. of M.I.E.-treated powder to effect a 1 per cent treatment of the untreated fluid or concentrate.

When additions of M.I.E.-treated fluid, concentrate, or powder were made at the hot well before forewarming, these additions were so made as to permit expressing the treatment on a fluid-fluid basis. Where concentrated M.I.E.-treated milk was used in treating untreated concentrated milk before filling and sterilizing, both concentrates were standardized to the same total solids content (26.2 per cent) and subsequently combined in various ratios. When the M.I.E-treated powders were employed in experiments with concentrated milk, the powder was first reconstituted to 26.2 per cent total solids and then combined with the untreated product on a concentrate-concentrate basis.

The fluid milks used in these experiments were taken from supply tanks containing about 20,000 lb. of average mixed milk. The initial experiments were conducted during the months of January to May, when milk is most unstable to heat.

All milks were forewarmed at 203° F. for 15 minutes with direct steam prior to condensing. The concentrated product was homogenized at 3,000 lb. per square inch in a Cherry-Burrell Viscolizer and then pumped over a surface cooler and cooled to about 40° F. After testing and standardizing to 26.2 per cent total solids, the product was canned in standard 14.5-ounce cans, sealed, and sterilized at 240° F. for 15 minutes in a case-size (45-can) Fort Wayne laboratory sterilizer. After cooling with cold water, the cans were removed for immediate examination and subsequent storage. In all experiments a sufficient number of cans of each type of treatment were prepared so that storage studies could be continued for 1 year.

In order to evaluate the M.I.E. treatment in terms of salt additions of comparable effectiveness, a number of direct comparisons were made. This necessitated the conducting of pilot trials on untreated concentrated milk to determine the optimum amount of stabilizing salt required to effect optimum stability during sterilization. Disodium phosphate ($Na_2HPO_4 \cdot H_2O$) was used as the stabilizing salt in all of these trials and is expressed in the conventional "ounces of salt per 1,000 lb. of concentrated milk" basis.

After sterilization and during storage at room temperature, the evaporated milks were examined for color, body, viscosity, pH, flavor, and butterfat separation. During storage the cans were not turned and samples were examined after 1, 2, and 4 weeks of storage. Subsequent examinations were made after 2, 4, 8, and, in some cases, 12 months of storage.

EXPERIMENTAL RESULTS

In the course of these investigations 16 separate experiments were conducted, several of the early trials being of an exploratory nature.

The first two trials involved the addition of concentrated M.I.E.-treated milk to the untreated concentrated milk before sterilization. Whereas the batches of concentrated milk in these two trials required 7.3 and 15.7 ounces, respectively, of disodium phosphate to produce optimum stability in the product, the addition of 2.5 and 6.0 per cent, respectively, of the M.I.E.-treated concentrate produced comparable results. Viscosity, body, pH, and flavor characteristics of the evaporated milks made by the two methods were very similar and compared favorably with the commercially available products.

Fluid Versus Concentrate Treatments

TABLE 1

A comparison of the stability of evaporated milks made with M.I.E. treatments in the hot well and after concentration (random trial)

Sample no.	Type of treatment	24 hr. after sterilization				
		pH	Viscosity, Borden-flow-meter (sec. at 60° F.)[*]	Body	Color	Flavor
1	Control, untreated	6.28	T.V.[†]	Curdy Grainy	Normal	
2	Control, salt-corrected[‡]	6.34	T.V.	Smooth Heavy	Normal	Normal
3	2.5% M.I.E.-treated fluid[§]	6.35	181	Smooth	Normal	Normal
4	5.0% M.I.E.-treated fluid	6.38	119	Thin	Normal	Normal
5	2.5% M.I.E.-treated concentrate[¶]	6.33	T.V.	Heavy Sl. grainy	Normal	Normal
6	5.0% M.I.E.-treated concentrate	6.37	586	Heavy Smooth	Normal	Normal

[*] A viscosity between 250 and 400 seconds was considered ideal 24 hr. after sterilization.

[†] T.V., too viscous to measure with Borden-flow-meter. Viscosities over 600 seconds are not dependable.

[‡] 15.7 ounces (per 1,000 lb. evaporated milk) disodium phosphate required for stability correction.

[§] Percentage of treatment made at hot well before forewarming.

[¶] Percentage of treatment made in concentrate before sterilizing.

The data presented in table 1 were taken from a representative trial in this series. The viscosity and body data show that only the equivalent of 2.5 per cent of the milk need be treated to correct the protein instability (requiring 15.7 ounces of disodium phosphate per 1,000 lb.) if the treated product is added to and forewarmed with the untreated milk. On the other hand, a 5.0 per cent (equivalent) treatment was required when the treated and untreated concentrates were combined just before sterilizing. In some of the other trials this difference was not so pronounced, and in one trial little difference was observed. Nevertheless, this series of trials showed a definite over-all advantage for the hot well treatment.

Evaporated milk frequently exhibits a very heavy or "livery" body after sterilization, but if not "grainy" the product "shakes out" into a smooth, desirable product. In this particular trial (table 1) the control salt-corrected product became thinner on storage and maintained desirable body characteristics for 1 year. The M.I.E.-treated products in this trial varied considerably in this respect because of the range of treatments. All M.I.E.-treated products except sample 5 were highly satisfactory evaporated milks. The 2.5 and 5.0 per cent treatments of the fluid milk (samples 3 and 4) at the hot well were somewhat more than would have been needed for ideal body characteristics. On the other hand, in the concentrate-treated samples (nos. 5 and 6), 2.5 per cent was insufficient to produce stability, as is evidenced by the grainy body. A 5.0 per cent treatment of the concentrate gave excellent results, and after 1 year in storage the quality of this sample was superior to any other in this experiment.

The Use of M.I.E. Milk Powder

As previously stated, the treatment of raw whole milk by the M.I.E. process was considered impractical because of mechanical difficulties in processing. Furthermore, there was no advantage in having the butterfat present during treatment, and in the powdered M.I.E. product the butterfat was additional bulk. Therefore, the last nine experiments were conducted employing M.I.E.-treated fluid, concentrate, and powdered skim milk products as stabilizing treatments for evaporated milk. Since the powdered products gave results comparable to those obtained with the same milk in fluid or concentrated forms and since the powder would be more practical for the small evaporated milk manufacturer, special emphasis was given to this phase of the problem.

Two trials conducted with M.I.E.-treated whole milk powder revealed that the powder was very effective in stabilizing evaporated milk. The powder was reconstituted to 26.2 per cent total solids and combined with the regular concentrated milk before sterilizing. One lot of the fluid was treated at the hot well before forewarming in order to compare these methods further. The results of one of these trials are presented in table 2.

A comparison of the concentrate (added just prior to sterilization) and fluid (hot well) treatments again reveals an advantage for the fluid method of M.I.E. stabilization. Had the percentage of fluid treatment been lower, a higher and more desirable viscosity would have resulted. In several trials where a lower-than-optimum viscosity was obtained with M.I.E.-treated milks at 240° F. sterilization, a desirable result was obtained by increasing the sterilizing temperature to 242–244° F. and holding for the conventional 15 minutes. In a number of comparisons where the M.I.E.- and salt-treated samples were sterilized at various temperatures, the body and viscosity characteristic of M.I.E.-treated samples appeared to be more stable to temperature change than the salt-corrected samples.

Other trials employing M.I.E.-treated skim milk powder gave very

TABLE 2

The use of M.I.E.-treated whole milk powder in stabilizing evaporated milk

Sample no.	Type of treatment	24 hr. after sterilization				
		pH	Viscosity, Borden-flow-meter (sec. at 60° F.)	Body	Color	Flavor
1	Control, untreated	6.29	T.V.†	Curdy Grainy	Normal	....
2	Control, salt-corrected*	6.28	235	Smooth	Normal	Normal
3	2.5% M.I.E.-treated concentrate	6.30	258	Smooth	Normal	Normal
4	5.0% M.I.E.-treated concentrate	6.33	145	Thin	Normal	Normal
5	2.5% M.I.E.-treated fluid	6.30	179	Good Sl. thin	Normal	Normal

* 10.4 ounces (per 1,000 lb. evaporated milk) disodium phosphate required for stability correction.

† Too viscous to measure.

similar results, so that a process involving the treatment of skim milk and its subsequent use as a fluid, concentrate, or powder seems most practical.

The Effect of the pH of M.I.E.-Treated Skim Milk on Its Efficiency as a Stabilizer of Evaporated Milk

Since increasing the pH of milk by the mere addition of a base has some stabilizing effect on the heat stability of milk proteins, the effect, if any, of

TABLE 3

The effect of pH adjustment of M.I.E.-treated skim milk on its stabilizing efficiency

Sample no.	Type of treatment*	24 hr. after sterilization				
		pH	Viscosity, Borden-flow-meter (sec. at 60° F.)	Body	Color	Flavor
1	Control, salt-corrected†	6.36	600	Very heavy Smooth	Normal	Normal
2	1.5% M.I.E. at pH 6.7	6.36	390	Heavy Good	Normal	Normal
3	3.0% M.I.E. at pH 6.7	6.35	187	Good	Normal	Normal
4	1.5% M.I.E. at pH 8	6.36	310	Heavy Good	Normal	Normal
5	3.0% M.I.E. at pH 8	6.37	100	Thin	Normal	Normal

* All M.I.E.-treated materials added at hot well before forewarming.

† 6.2 ounces disodium phosphate required for stability correction of 1,000 lb. evaporated milk.

the somewhat increased pH of the M.I.E.-treated milk solids was determined. A portion of a lot of M.I.E.-treated skim milk was re-treated in a hydrogen exchanger so as to adjust the pH from 8.0 to 6.7. The two lots were dried by the spray method and the powders used in stabilizing evaporated milk. Two trials were made on successive weeks when quite unstable winter milk was available. Since little butterfat was present in the powder, the "percentage of treatment" was on a solids-to-solids basis.

The results obtained in both of these trials were very similar and confirmed the fact that the product at pH 8.0 had only a slight advantage over the one in which the pH was adjusted back to 6.7 (table 3). The data also show that the use of the unadjusted material has very little effect on the pH of the finished evaporated milk. This observation supports the data presented in tables 1 and 2, as well as the data obtained in numerous other trials.

The viscosity of sample 1 (control, salt-corrected) was very high after sterilization. It became much less viscous after 1 week in storage and, together with samples 2 and 4, finished a 1-year storage period in very fine condition. Samples 3 and 5, which actually were overstabilized, were thin and showed considerable fat separation (creaming) after 4 months and 1 month, respectively.

The Application of the M.I.E. Treatment to Evaporated Milks with Higher-Than-Normal Solids Content

As the percentage of total solids in evaporated milk is increased, the tendency toward coagulation during sterilization becomes progressively more pronounced. Moreover, evaporated milk containing more than 26.2 per cent total solids will exhibit darker color, more astringent flavor, lower pH, and greater tendency toward mineral deposits after sterilization.

A series of trials were conducted in which the total solids content of evaporated milks was varied from 26.2 to 34.0 per cent. These trials were made in early May when milk was becoming more stable to heat. At this particular time the conventional 26.2 per cent total solids product could be corrected with 2 ounces or less of disodium phosphate per 1,000 lb. of concentrate. In one trial where the concentration was carried to 34.0 per cent total solids, 47 ounces of disodium phosphate per 1,000 lb. of product was required to produce stability in the sterilized product. A parallel trial in the same experiment, involving the M.I.E. treatment of the fluid milk at the hot well, showed that by treating 5.0 per cent of the product, comparable viscosity and body characteristics could be effected.

Two other comparisons at somewhat lower concentrations demonstrated that a comparably small percentage of M.I.E.-treated product was highly effective in controlling the coagulating tendencies of high-solids evaporated milk.

This particular phase of the study is being given further consideration and will be reported upon in the near future.

The Effect of M.I.E. Treatment on the Calcium, Phosphorus, and Ash Content of Evaporated Milk

Calcium and phosphorus are very important nutritional factors in milk. Any process or treatment that would disturb either the concentration or particularly the ratio of these constituents in milk would be undesirable. Therefore, it was of interest to demonstrate the magnitude of change in the concentration of these elements in evaporated milk as a result of M.I.E. treatment.

The data presented in table 4 show the calcium, phosphorus, and ash analyses of evaporated milks made in 9 of the 16 experiments. The data given in each case are for only the optimum M.I.E. and disodium phosphate treatments.

TABLE 4

The calcium, phosphorus, and ash content of M.I.E.- and disodium phosphate-corrected evaporated milks (all data corrected to 26.2 per cent total solids)

Expt. no.	Type of stabilizing treatment	Amount of treatment	Calcium (%)	Phosphorus (%)	Ash (%)
1	Disodium phosphate	7.3 oz.	0.2523	0.2220	1.546
	M.I.E.	5.0%	0.2438	0.2203	1.530
4	Disodium phosphate	8.3 oz.	0.2541	0.2125	1.529
	M.I.E.	2.5%	0.2509	0.2094	1.526
5	Disodium phosphate	18.7 oz.	0.2508	0.2093	1.554
	M.I.E.	5.0%	0.2401	0.1998	1.468
6	Disodium phosphate	10.4 oz.	0.2353	0.2034	1.487
	M.I.E.	2.5%	0.2532	0.1977	1.507
8	Disodium phosphate	8.3 oz.	0.2509	0.2062	1.521
	M.I.E.	3.0%	0.2535	0.1985	1.469
9	Disodium phosphate	2.0 oz.	0.2555	0.2098	1.529
	M.I.E.	0.5%	0.2551	0.2023	1.533
10	Disodium phosphate	2.0 oz.	0.2430	0.2053	1.509
	M.I.E.	0.5%	0.2452	0.2455	1.505
11	Disodium phosphate	10.4 oz.	0.2505	0.2275	1.537
	M.I.E.	2.0%	0.2518	0.2173	1.547
12	Disodium phosphate	6.2 oz.	0.2438	0.2064	1.474
	M.I.E.	1.5%	0.2477	0.2092	1.496

The calcium content of the evaporated milks was altered only insignificantly by the M.I.E. treatment. Similarly, the ash content is essentially the same. The somewhat higher values found for phosphorus in the salt-corrected samples undoubtedly are due to the added disodium phosphate. From the data presented here it can be concluded that the amount of M.I.E. treatment required to stabilize evaporated milk has no significant effect upon the calcium, phosphorus, and ash content of the finished product.

DISCUSSION

Treatment of a small percentage of a given lot of milk by the M.I.E. technique is an effective means of stabilizing evaporated milk. The finished product made with M.I.E.-treated milk compared favorably with that obtained with the conventional "salt correction" method and was particularly outstanding in cases where very unstable milk was concerned. After storage for 1 year, evaporated milks made by the two methods were found to be essentially the same with respect to color, flavor, viscosity, pH, and fat separation. In no case was it found impossible to stabilize the milk by either the salt or the M.I.E. method during the season from January to May when milk usually exhibits its maximum instability.

M.I.E.-treated fluid milk and the same product condensed or dried were equally effective in stabilizing evaporated milk against coagulation during sterilization. Treatment of the fluid at the hot well before forewarming generally was more effective than the method of treating the concentrate just before filling and sterilizing.

Normal M.I.E.-treated milk exhibits a pH of approximately 8.0. By restoring the pH to approximately normal (6.7) with a hydrogen exchanger, the effectiveness of the product was altered very little, thereby demonstrating that the somewhat elevated pH of the product contributes little to its stabilizing effect. The use of unadjusted M.I.E. products in concentrations required to stabilize evaporated milk had essentially no effect on the pH of the finished product.

The stabilization of evaporated milk with M.I.E.-treated milk has no significant effect on the calcium and phosphorus content of the product. The amount of treatment required to stabilize most evaporated milks is so small that its effect scarcely can be detected by conventional calcium analyses.

Preliminary trials demonstrated that where almost prohibitive amounts of disodium phosphate were required to stabilize high total solids (34.0 per cent) evaporated milk, a treatment of only 5.0 per cent of the original fluid effected a desirable result.

The mechanism by which the M.I.E. principle acts in the stabilization of evaporated milk could not be determined definitely from the data taken in this study. However, since instability of evaporated milk generally is attributed to excess calcium and magnesium ions, the re-establishing of a more favorable ionic equilibrium by the physical removal of these excess ions by M.I.E. treatment appears to be the most logical explanation.

CONCLUSIONS

1. The mineral-ion exchange process as employed in these experiments with evaporated milk is capable of stabilizing the product against coagulation during sterilization at 240° F. for 15 minutes.

2. This process has been found effective in stabilizing milks exhibiting a wide range of instability to heat.

3. The addition of optimum quantities of mineral-ion exchange-treated

milk to evaporated milk before sterilizing imparts characteristics similar to those obtained when the product is stabilized with salts.

4. Under average conditions where a product requires 2 to 6 ounces of stabilizing salt per 1,000 lb. of concentrate to produce stability, the same correction can be effected by treating from 0.5 to 2.0 per cent of the original milk by the mineral-ion exchange process.

5. The addition of the mineral-ion exchange-treated milk at the hot well before forewarming produces somewhat greater efficiency of stabilization than addition later.

6. The mineral-ion exchange-treated milk can be used as a fluid, concentrate, or powder with almost equal effectiveness.

7. Preliminary trials suggest that the mineral-ion exchange method of stabilization may be very effective in the manufacture of high total solids (32–34 per cent) evaporated milk.

8. The utilization of this process in the manufacture of evaporated milk has only an insignificant effect on the calcium and ash content of the product.

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SEASONAL VARIATIONS IN HOURS OF DAYLIGHT ASSOCIATED WITH FERTILITY LEVEL OF CATTLE UNDER NATURAL BREEDING CONDITIONS

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Losses due to sterility or low fertility amongst livestock in North America amount to millions of dollars annually. Though this fact is recognized by most workers in the field, controlled investigations have not yielded evidence to suggest that eliminating these losses is an easy matter soon to be achieved (2).

Sterile animals are identified readily in most dairy herds, but inefficient reproducers may be more difficult to expose and, in the aggregate, may contribute fully as much to monetary loss. The rapid growth of artificial breeding has served to emphasize the importance of the differences in relative fertility among dairy cattle and to focus attention on this problem.

From the records accumulating on thousands of cattle in the New York artificial breeding program it was believed that it might be possible to obtain evidence on the causes of the varying levels of fertility observed. The records are obtained from herds operated under a variety of conditions in all agricultural counties of the State. The data first were studied with the objective of determining the effect of various factors on fertility results. Certain inseminating practices and regional problems were studied. However, it was evident immediately that certain major factors influenced fertility level. These factors, unless thoroughly understood, might obscure other influences, or bias data used for other studies.

The first factor of major importance was the age of the cattle concerned. Data on that problem were published by Tanabe and Salisbury (31). Later Mercier and Salisbury (20) studied what then appeared to be the second major influence, that of season on fertility level of dairy bulls; highly significant differences between months in fertility level were found. Earlier Salisbury (27) had found seasonal variations in semen quality which were not reflected in variations in fertility level. Others (8, 12, 15, 21, 23, 24, 30) had noted a seasonal trend in fertility level of cattle, which they attributed largely to variations in temperature. The results of these studies were so diverse that no general conclusions could be drawn.

Recently, Mercier (19) reviewed the published data concerning the monthly fluctuations in fertility level of cattle. From his review and his

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own data he suggested that the seasonal fluctuations observed were not random variations, but followed definite patterns associated with climatic factors, of which variations in length of daylight played an important part. Previously, as mentioned above, the seasonal fluctuations in fertility level of cattle had been attributed largely to variations in temperature. However, it has been shown that light is the primary agent regulating the breeding behavior of many species of birds and lower mammals. This subject has been reviewed at length by other investigators (3, 4, 6, 18, 26). Evidence is yet to be produced to show that light does not influence fertility level in cattle.

The authors recognized that the definite experimental demonstration of the influence of light variations on fertility level of cattle would involve an enormous outlay of time and effort. In an experiment of this kind, more animals would be required to produce valid conclusions than usually are considered necessary. For direct proof of the theory it would be necessary to show that fertility level was modified by light manipulations. To demonstrate the reasons for such modifications of the fertility level by light manipulations, the morphological and physiological changes in the primary reproductive organs, as well as the hormone balance of the entire body, would have to be studied minutely.

As adequate facilities were not available for direct experiments on the question, it was felt that an indirect approach might reveal definite facts from which the importance of light variations on fertility level could be assayed. Thus, a detailed statistical study was made of Mercier's data (19), and additional evidence was obtained from the records of the New York Artificial Breeders' Cooperative, Inc.

The present report deals with a study of the conception rate of cattle under natural breeding conditions in Eastern Canada. It was undertaken because the authors felt that the possible detrimental effect of extreme temperatures on reproduction would be minimized because of the cool Canadian summers and the wintering of cattle in warm barns. On the other hand, they believed that the effect of light, if involved, would be emphasized because of the extreme length of the summer days and the shortness of the winter days.

MATERIALS AND METHODS

Three main points considered in the selection of herds to provide breeding records for this study were the latitude at which the farms were situated, the similarity in management practices, and the reliability of the available breeding records.

The purebred herds of the three following institutions, containing cows of the Ayrshire, Holstein, and beef and dual-purpose Shorthorn breeds, were selected: The College of Agriculture of MacDonald, Quebec, and the Experimental Stations of Ste-Anne-de-la-Pocatiere, Quebec, and Kapuskasing, Ontario. Only a few females were brought into the herds during the period covered in this study, 1919–1944. In all herds there was a tendency to use sires of various ages, and no close-breeding systems were followed. Abortion waves occurred in two herds. Before 1935, reactors were eliminated; after that, the policy was to isolate females that aborted. In order to avoid bias due to these troubles, all records used were from cows which conceived prior to the fifth service.

The farms were located almost exactly 2° of latitude apart, one at 45°, one at 47°, and the other at 49°. These differences had little effect on monthly mean daylight hours, the northernmost station having approximately one-half hour less of daylight during January and the same amount more during June. As a consequence the monthly average number of daylight hours and hours of sunshine varied somewhat from station to station, but this variation was unimportant in comparison with the extreme change throughout the year. The monthly mean number of daylight hours varied from 8.2 hours in December to 16.0 hours in June. At no time during the year did the mean monthly outside temperature at any station reach 70° F., which is well below the point of hyperthermal rise in body temperature.

The breeding records for each herd were sorted by month of service, and the number of successful services determined for each month. The data were tabulated as the per cent of successful services and were so used in this investigation. However, in order to make the results comparable with others published, they are expressed as services per conception.

The monthly per cent of successful services for each herd was considered as a single observation. Months were grouped into seasons: winter months being December, January, and February; spring months, March, April, and May; summer months, June, July, and August; and fall months, September, October, and November. Analyses of variance and covariance techniques, as described by Love (17), were used to colligate the evidence. In the first case the per cent of successful services was used, while in the second, the independent variable was the number of services and the dependent variable the number of conceptions. In the use of each statistical technique no significant herd × season interaction was observed. Thus, in each analysis the entire remainder including the primary interaction variance was used for testing the statistical significance of "herds" and "seasons".

RESULTS

The data grouped by season and herd are presented in figure 1. No significant differences in fertility level between herds were found by either method of analysis, but both methods showed a significant difference in fertility level between the seasons at the 5 per cent level of probability. These data show that fertility level as measured by the per cent of successful services was highest in summer and lowest in the winter and spring in these

Canadian herds. A study of the monthly per cent of successful services for the three herds suggested a definite trend. The poorest conception rate was obtained during the month of February and the best during the month of July, some time after the shortest and the longest day of the year, respectively.

For comparative purposes a survey of the number of services per conception in cattle obtained at various locations in the Northern Hemisphere is presented in table 1. The information published by Clapp (9) for the Cornell University dairy herd shows the same trends as do the data reported here and by Mercier (19). Except for the data of Dawson (10), Liang [Phillips *et al.* (23)], and Seath *et al.* (30), which are largely from the southern portion of the Hemisphere, there is a tendency for the number of services per conception to decrease from February on through the spring until the days begin to shorten and the hot weather of summer supervenes.

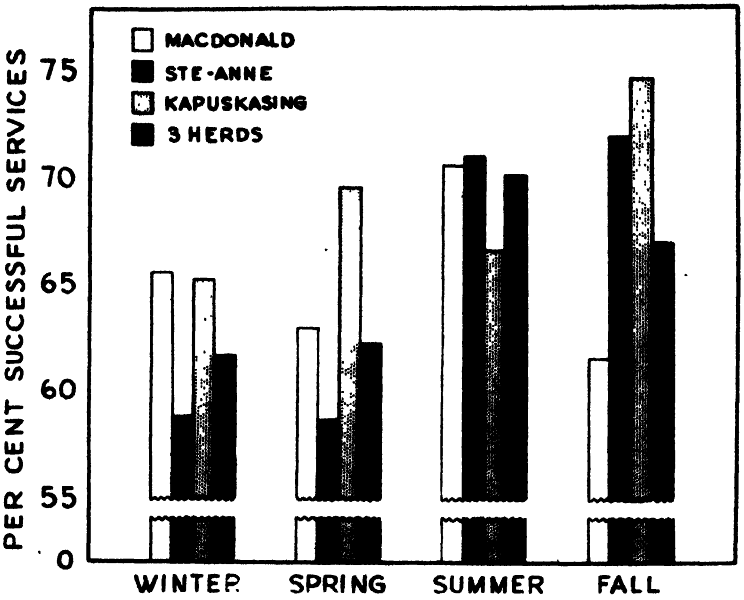

FIG. 1. Seasonal variations in fertility level of three herds in Eastern Canada.

The observed trend suggested that increased length of day or increased temperature, up to an optimum temperature, might beneficially influence reproduction in cattle. In Mercier's data (19) no decrease in fertility level during the summer was observed. In fact, the variations in fertility level seemed to vary directly with the length of daylight, the hours of sunshine, and the outside temperature, there being a lag of some time before the maximum effect was reached.

The relationship between each of these climatic factors and the average fertility level of the three herds was determined. To get an idea of the

TABLE 1

Seasonal variation in conception rate of cattle under natural conditions
(services per conception)

Month	Dawson (10) 8 states	Morgan & Davis (21) Nebr. 1896–1934	Erb *et al.* (12) Ind. 1920–'40	Hilder *et al.* (15) Md. 1919–'42	Clapp (9) N. Y. 1929–'45	Mercier (19) E. Canada 1919–'44	Phillips *et al.* (24) Md. 1935–'42	Seath *et al.* (30) La. 1923–'40	Liang (23) S. China
Jan.	2.50	2.08	1.54	2.57	2.18	1.51	1.93	Winter	Dec.–Feb.
Feb.	2.34	2.25	1.69	2.81	2.29	1.67	1.89	2.5*	1.71
March	2.45	2.46	1.41	2.80	2.05	1.54	1.80	1.5†	March–May
April	2.26	2.16	1.53	2.65	2.03	1.56	1.68	Spring	1.96
May	2.54	2.20	1.35	2.64	1.99	1.66	1.71	2.8*	
June	2.63	2.10	1.47	2.36	1.96	1.43	1.82	1.9†	June–Aug.
July	2.31	2.07	1.66	2.94	1.90	1.41	2.40	Summer	2.37
Aug.	2.48	2.78	1.72	2.88	2.20	1.43	2.45	3.4*	
Sept.	2.56	2.85	1.60	2.82	2.11	1.46	2.38	2.4†	Sept.–Nov.
Oct.	2.42	1.99	1.60	2.52	2.13	1.49	1.98	Fall	1.66
Nov.	3.08	2.09	1.57	2.64	2.26	1.50	1.85	2.7*	
Dec.	2.50	1.94	1.64	2.52	2.26	1.52	1.82	1.4†	
Av.	‡$\frac{2982}{1197} = 2.49$	$\frac{2979}{1375} = 2.16$	$\frac{1440}{922} = 1.56$	$\frac{9181}{3439} = 2.67$	$\frac{4250}{2019} = 2.11$	$\frac{3543}{2322} = 1.53$	$\frac{1135}{584} = 1.94$	. . .	$\frac{795}{430} = 1.85$

* = Expt. herd.
† = Univ. herd.
‡ = $\frac{\text{Services}}{\text{Conceptions}}$.

length of the apparent lag effect, the correlation coefficients between the per cent of successful services for one month and the average monthly values for temperature, length of day, and number of hours of sunshine for the month corresponding to and of the first, second, third; and fourth month prior to mating were calculated. The correlation coefficients are shown in table 2.

The relationship between fertility level and each of the three climatic factors was approximately of the same magnitude. The correlation coefficients for the second month prior to mating, in general, were the highest. As hours of sunlight are a function of the length of day, and as Olson (22) has reported that lack of direct sunshine had no measurable effect on reproduction in cattle, the authors have eliminated this item from further consideration. Partial correlations for the second month prior to mating, using the individual observations for each herd, indicated that length of daylight and not temperature change was the more important factor associated with the conception rate under Canadian conditions. The partial correlation coefficients were 0.352 and 0.017, respectively, the first being significant at the 5 per cent level of probability. The results suggest that longer daylight has a beneficial effect on the conception rate of cattle in Eastern Canada.

TABLE 2

Correlations between the conception rate and the monthly averages for climatic factors for the month corresponding to and the months prior to mating

Correlations between fertility level and:	Months for climatic factors				
	Corresponding	Prior to mating			
		First	Second	Third	Fourth
Temperature	0.588*	0.732†	0.692†	0.441	0.220
Day-length	0.275	0.604*	0.731†	0.668†	0.460
Daily sunshine hours	0.346	0.573*	0.697†	0.603†	0.388

* Significant at the 5% level of probability.
† Significant at the 1% level of probability.

That this observation may have wider application is indicated by other evidence. In England, Hammond (14) and Sanders (29) observed that cows calving in spring and bred in summer, especially in the northern districts of the country (14), required less time to get in calf than those calving in fall and bred in winter. Plum and Lush (25) found that heifers in Iowa bred in spring and summer calved at an earlier age than those bred in late fall and winter. In a similar study of the age of Ayrshire heifers in the United States calving for the first time during 1942–1945, Salisbury and Mercier (28) observed the same trend. Furthermore, in their data, comparison of the ages of the heifers calving in the states below the 40° of latitude, none of which are in the extreme South, and those calving in the states north of that parallel showed that the monthly average age at first calving varied more for the group calving in states north of the 40° of latitude than for the group calving in states south of that latitude. Huntington (16) has observed a similar increase in the amplitude of seasonal conception curves in human beings in the United States and Canada as the population areas studied shifted from the Equator towards the North. It would appear that at high latitudes heifers as well as mature cows are somewhat less fertile during periods of the shortest length of day and, as a consequence, conception and calving are delayed.

DISCUSSION

Dawson (10), studying the fertility of aged bulls used at various locations in the United States, found that the relative fertility of sires used at southern stations was 36 per cent as compared to 49 per cent for sires used at northern and western locations. Seath *et al.* (30) in Louisiana and Liang [Phillips *et al.* (23)] in southern China attributed the poor results secured during summer and spring (see table 2) to high environmental temperature. Although this interpretation may explain satisfactorily the contrasting seasonal difference between their results and those of the authors, it does not explain adequately the rather uniformly low monthly conception rate of aged sires in the south of the United States (10).

It is known that the seasonal variation in day-length becomes less and less, the winter days being relatively longer and the summer days shorter, as one approaches the Equator. For instance, the day-length averages for the months of June and December are, respectively, 16.0 and 8.2 hours in Eastern Canada (1) and 14.1 and 10.3 hours in Louisiana (33). The lesser variation in length of daylight may account somewhat for the relatively good winter results reported at low latitudes (10, 23, 30).

That level of nutrition is probably not a controlling factor in the increased breeding efficiency observed as spring advances is indicated by the fact that in the north the gradual change is underway before pasture becomes available. Also, the evidence suggests that confining cows in stanchions is not responsible for the lower breeding efficiency observed in winter. Webster (35) from New Zealand reported that the majority of barren cows kept on pasture the year around bred successfully, most of the time at the first attempt, when carried over the winter to the following spring.

Though the action of light on reproductive activity of vertebrates is not entirely clear, it is known to act on the pituitary and possibly on other areas of the midbrain through the eye, retina, and the optic nerve (3, 4, 6, 18, 26). Fiske (13) has shown that light stimulates the pituitary of the female rat to produce and release follicle-stimulating hormone. Conversely, lack of light favors an increase in the production of luteinizing hormone.

Since the relationship of temperature to the reproductive processes is not thoroughly understood, the effect of high temperature on the semen production of males may be only a secondary effect rather than a primary one. There is direct evidence that temperature affects the function of the thyroid gland in the rat (11) and in the ram (7) and also the gonadotropic potency of the anterior pituitary in the ground squirrel (36). Furthermore, it has been shown that in the rat (32) the pituitary controls the rate of formation and the release of thyroxin into the circulation, and that it acts on the gonads of certain species of fish (34) through the thyroid. Seasonal variations in the amount of thyrotropic hormone in the blood of cocks and ducks, associated with changes in the activity of the thyroid and the testis, were reported by Benoit and Aron (5).

While these reports strongly suggest that the seasonal variations in reproductive efficiency reflect primarily pituitary and, secondarily, thyroid activity, more evidence is needed before definite conclusions may be drawn.

The results of research published by others and the work reported here lead the authors to conclude that light plays an important role in controlling the breeding efficiency of cattle. Obviously, further studies should be made to determine any specific effect of light and temperature on reproductive activity of cattle of various ages and to modify, if possible, these two environmental factors or to change management practices in order to improve conception rate, especially of cows bred to calve in the fall.

SUMMARY

In a study of three herds of cattle located at different latitudes in Eastern Canada, the lowest per cent of successful services was obtained during winter and spring and the highest during summer and fall. The differences in fertility level between herds were not significant statistically but those between seasons were significant at the 5 per cent level of probability.

The average monthly conception rate of the three herds was significantly correlated with the monthly average length of daylight, there being a lag of approximately 1 to 2 months before the maximum effect was reached. Temperature changes had no measurable direct effect on fertility level under the existing conditions.

The authors believe that at high latitudes variations in length of daylight measurably influence the fertility level in cattle.

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THE RELATION OF PER CENT TOTAL SOLIDS IN SEPARATED MILK AND PER CENT FAT IN WHOLE MILK TO ATMOSPHERIC TEMPERATURE

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During recent years, the general use of continuous processing methods and various types of automatic process controllers has reduced or eliminated many elements responsible for deviations from quality standards. This has resulted in a greater appreciation of the significance of variations in the composition of milk both on a day-to-day and on a seasonal basis.

Daily variations in the fat test of milk also are of interest to the dairyman who occasionally may have special fat tests made during a period of only a few days. Dr. E. W. Bird of Iowa State College called the author's attention to the fact that although numerous reports have been published establishing several reasons for variations in fat test, the significance of day-to-day variations in atmospheric temperature has not been stressed. The data presented in this report are intended, therefore, to demonstrate that variations in atmospheric temperature from one day to the next are sufficiently significant to be of major importance to the plant operator and the dairyman.

LITERATURE

Several studies have shown that the temperature affects the fat percentage of milk. Generally, this conclusion has been reached on a seasonal basis rather than on a day-to-day basis. During March and April, 1922, however, Ragsdale and Brody (3) compared the average fat test of the milk from ten cows over a 24-hour period with the mean atmospheric temperature of the same 24 hours and concluded that there is roughly about 0.2 per cent increase in fat for each 10° F. decrease in temperature.

Hays (2), working with Ragsdale and Brody, carried out a more comprehensive study. Daily data for fat tests and air temperatures were collected for 258 days. The environmental temperatures ranged from 85.5 to 24.5° F., and the average fat tests ranged from 3.171 to 3.600 per cent. This increase in fat test amounted to 0.079 per cent for each 10° F. lowering of the temperature. However, Hays did not consider these results as significant as those obtained on two Jersey cows which were maintained for 4- to 8-day intervals in controlled temperature rooms. The result of these trials indicated a rise of 0.189 per cent fat for each 10° F. lowering in temperature.

Regan and Richardson (4) found that at 80 or 85° F., depending upon the breed, a pyrexial point was reached where animals no longer were able

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to maintain heat balance. This probably explains some of the adventitious results secured by Hays on his trials with two Jersey cows. Regan and Richardson further noted that above the pyrexial point many changes were observed in the chemical constituents of milk, such as lowering of casein and solids-not-fat, increase in fat and pH, depression of the freezing point, a longer rennet coagulation time, and lower per cent volatile acids and higher per cent unsaturated components of the butterfat secreted.

Gaalaas (1) reported a definite relationship between the body temperature of the cows and the air temperature. The correlation coefficient was found to be + 0.57 ± 0.0079 for a total of 3,298 readings of body temperature. The correlation coefficient for respiration rate and air temperature was + 0.77 ± 0.0048.

Seath and Miller (5), studying the effects of both temperature and humidity on the body temperature and respiration, found that partial correlations between air temperature and body temperature (humidity held constant) were + 0.674 for 1944 and + 0.534 for 1945 on 36 Holsteins and 16 Jersey milking cows. The effect of humidity on body temperature was of minor importance. Partial correlation coefficients for air temperature and respiration (humidity held constant) were + 0.748 for 1944 and + 0.353 for 1945. Humidity again was of minor importance.

Previous reports, therefore, indicate that: (a) a general relationship exists between air temperature and the fat test of the milk; (b) after the pyrexial point has been reached, significant changes occur in the composition of the milk; and (c) a definite correlation exists between both body temperature and respiration rate of the cow and air temperature, with humidity playing an insignificant role.

METHODS

Total solids were determined by the Mojonnier method on a composite of several samples of separated milk taken at hourly intervals during the time of operation. The per cent total solids of the separated milk, rather than the per cent total solids in whole milk minus the per cent fat, was used as an index of the nonfat solids, since a more representative sample could be obtained. Since it was determined statistically that random sampling of one-seventh of the total amount of milk received would result in the desired accuracy, this amount of milk was cooled, held in storage tanks, agitated thoroughly, and sampled. Babcock tests were made on each of the several samples resulting, and these tests were averaged to obtain a figure representative of the daily fat test.

Mean daily temperatures were secured from the United States Weather Bureau located in Springfield, Missouri. Since all the milk is received within a radius of 40 miles of Springfield, it is felt that the mean daily temperature at Springfield is a fair representation of the temperature existing in this area. There is no conclusive evidence to support this statement, but it is known that weather stations located between 50 and 70 miles away never have recorded temperatures at great variance with those recorded at Springfield.

Another factor very slightly affecting the accuracy of the figure given as mean daily temperature is that the hours used for arriving at this figure do not coincide with general milking times. Mean daily temperatures are based on hourly readings from 1:20 a.m. to 12:20 a.m. of the following day. It would have been more desirable for the purposes of this study to have the hours begin with 7:00 a.m. and end with 6:00 a.m. of the following day. This discrepancy of 5 or 6 hours, however, may be significant only during sharp changes in atmospheric temperature, which are rare. Very little difference between mean daily temperatures calculated by the two methods was found when hourly temperatures were recorded for a 10-day period. It should be emphasized that the mean daily temperature is the temperature of the day before the milk was received at the plant, *i.e.*, the air temperature at the time the cows were secreting milk.

The statistical methods used are those described by Snedecor (6). The mean daily temperatures were correlated with the mean daily fat tests of the whole milk received and of the mean daily total solids of the separated milk. A period of one month was chosen for convenience. Shorter periods would tend to reduce the effect of stage of lactation and would show less variation in the value of the correlation coefficient. Longer periods would increase the effect of stage of lactation and other factors affecting the composition of milk.

About 90 per cent of the cows in this area are Jerseys. As reported by Seath and Miller (5), this breed is more tolerant than the Holstein to high temperatures. Since the climate is temperate, cows are kept in barns only occasionally during short periods of severe cold. The milk received at the plant represents the combined volume of about 6,000 herds.

RESULTS

Table 1 shows the correlation coefficient, the regression coefficient, and the deviations from regression between the mean daily total solids and mean daily temperature for monthly intervals for the year 1946. Table 2 shows the correlation coefficient, the regression coefficient, and the deviations from regression between the mean daily temperature and the mean daily per cent fat in the whole milk for monthly intervals for the year 1946.

The results of this statistical study indicate that there is a relationship between atmospheric temperature and the per cent total solids in separated milk during 1946. The values of the correlation coefficient range from -0.062 to -0.758. The results also indicate a significant relationship between atmospheric temperature and the per cent fat in whole milk for the same year, the values ranging from -0.258 to -0.838. The fat test appar-

TABLE 1

The relation between the mean daily total solids in separated milk and the mean daily atmospheric temperature for 1946, calculated for monthly intervals

Month	Correlation coefficient	Regression coefficient	Deviation from regression
January	- 0.062	- 0.0010	0.092
February	- 0.367	- 0.0034	0.087
March	- 0.290	- 0.0035	0.084
April	- 0.329	- 0.0048	0.112
May	- 0.403	- 0.0045	0.061
June	- 0.758	- 0.0119	0.077
July	- 0.456	- 0.0080	0.068
August	- 0.721	- 0.0119	0.083
September	- 0.731	- 0.0148	0.079
October	- 0.342	- 0.0059	0.121
November	- 0.191	- 0.0042	0.147
December	- 0.663	- 0.0069	0.081
Mean	- 0.4427	- 0.00673	0.091

ently is affected to a greater extent by temperature than is the total solids content.

For the year 1946 there was an increase of 0.067 per cent in the total solids of the separated milk and an increase of 0.093 per cent fat in the whole milk for every 10° F. decrease in atmospheric temperature over a range from 34.8 to 78.8° F. in the Springfield, Missouri, area.

DISCUSSION

Since the fat test of all the milk received at this plant shows a definite negative correlation with the mean daily temperature, one may safely assume that the test of the milk of individual herds will show a similar change with temperature, and that the test of the milk from individual cows in each herd also will vary with the temperature. Actually, this has not been

TABLE 2

The relation between the mean daily fat in whole milk and the mean daily atmospheric temperature for 1946, calculated for monthly intervals

Month	Correlation coefficient	Regression coefficient	Deviation from regression
January	- 0.838	- 0.0105	0.091
February	- 0.673	- 0.0062	0.073
March	- 0.612	- 0.0058	0.068
April	- 0.629	- 0.0107	0.086
May	- 0.619	- 0.0060	0.060
June	- 0.757	- 0.0076	0.063
July	- 0.258	- 0.0036	0.039
August	- 0.770	- 0.0180	0.103
September	- 0.769	- 0.0183	0.094
October	- 0.742	- 0.0087	0.075
November	- 0.570	- 0.0085	0.095
December	- 0.642	- 0.0075	0.111
Mean	- 0.6566	- 0.00926	0.0798

found true in all cases, but it generally was found true during May and June, 1947, when a study was made of the fat test of the milk of 75 patrons.

The low correlation coefficient (–0.258) obtained for fat in July may be due to several factors. In July, 1946, the temperature was uniform for an extended period. Under this condition, the effect of the stage of lactation is likely to be more pronounced. Furthermore, in hot weather, cows may be shaded to a variable degree, which will have a variable effect on their body temperature. Finally, when the atmospheric temperature is uniform, the errors of sampling and testing are likely to affect the correlation coefficient to a greater degree.

Tracy and Tuckey (7) and others have reported data showing day-to-day changes in the fat test of individual patron's milk as received at milk plants. These results indicated that a majority of the samples tested vary throughout a 7-day test period from 0.3 to 0.75 per cent, and some samples vary over 2.5 per cent. Unpublished results of similar studies at this plant lead to the same conclusion. Although Tracy and Tuckey are inclined to attribute this variation to malpractice, it is probable that changes in air temperature largely are responsible.

The effect of air temperature should be recognized by dairymen and testing associations, who often judge the fat production of a cow on the basis of the results of a few days' testing. Obviously, these results may or may not reveal a true production record. Composite sampling or daily testing for several days is the most desirable way of arriving at reliable results.

The nonfat solids (as measured by the total solids in the separated milk) have not been of interest to the dairyman. However, in whole milk plants where standardization of the fat test is carried out, as in the case of cheese plants, the variation in the nonfat solids may have some significance, and the recognition of causes for this variation may be of value.

SUMMARY

The results of this study indicate that a definite relationship usually exists between the fat test of the whole milk received at the plant, the total solids of the separated milk, and the air temperature in the Springfield, Missouri, area.

The correlation coefficients between mean daily total solids of separated milk and mean daily temperature ranged from – 0.062 to – 0.758 and averaged – 0.443 for the year 1946, using monthly intervals.

The correlation coefficients between mean daily fat test of the whole milk as received and the mean daily temperature ranged from – 0.258 to – 0.838 and averaged – 0.657 for 1946.

There was an average increase of 0.067 per cent total solids of the separated milk for each 10° F. decrease in atmospheric temperature.

There was an average increase of 0.093 per cent fat in the whole milk for each 10° F. decrease in atmospheric temperature.

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FURTHER STUDIES OF THE PROCESSES INVOLVED IN THE PRESERVATION OF CAROTENE IN DEHYDRATED ALFALFA AND CEREAL GRASSES[1]

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In an earlier paper (1) it was pointed out, as also has been done by others, that a dehydrated alfalfa or cereal grass having a water content of 6–10 per cent lost carotene fairly rapidly under ordinary conditions of storage. After addition of water to a level of 15–20 per cent total and storage in waxed containers, these same products lost none of their carotene. In other words, under airtight storage and with the proper water content, the oxygen present gradually is used up and displaced by carbon dioxide. In the absence of oxygen, the carotene is preserved completely. As will be pointed out, these plant tissues with a water content of 6–10 per cent slowly respire, using up the oxygen and producing carbon dioxide, but the rate is so slow that carotene losses can occur before the oxygen present has been consumed completely. Higher water levels, such as 12–20 per cent, increase the rate of respiration, and the oxygen is used up much faster. When the product is stored in receptacles impervious to oxygen, conditions for carotene preservation readily are established.

To study the relation of water level to the rate of carbon dioxide production, new experiments were inaugurated. The type of drying to which the material was subjected also was studied.

EXPERIMENTAL

Two types of driers are employed extensively in this country for the preparation of commercial dehydrated alfalfa and cereal grasses. The one more commonly used appears to be the Arnold Drier (Heil) made by the Heil Company of Milwaukee, Wis. In this drier the furnace gases enter at a temperature of about 1850° F. (1010° C.) and leave the dehydrator at about 250° F. (121° C.). The product is in the drier from 1.5 to 5 minutes. The product produced is green in color, and grasses usually are brighter green than alfalfa, a phenomenon no doubt dependent upon the initial chlorophyll content. Quite surprising is the fact that the chlorophyll is retained at all, but it is more than probable that the organic structures of the plant materials never reach the temperature recorded for the ingoing and outgoing gases. (See sample 1 in table 1.)

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The second drier used is the Fulmer Drier. In this dehydrator a temperature of about 290° F. (143° C.) is used and the material is in the drying tunnel approximately 25 minutes. The time required for dehydration is dependent upon the initial moisture content and may be as much as 10 to 15 minutes longer when the material carries about 80 per cent water. This product also retains its green color (sample 2).

TABLE 1

Carbon dioxide production in dehydrated alfalfa and oat grasses

	Total water	CO_2	O_2
	(%)	(%)	(%)
Sample 1 Alfalfa (Caple Bros.) Fulmer Drier. Entering gases 143° C., 30 min. Analyzed after 5 days' storage at room temperature. Original water 6%.	6.0	1.0	18.5
	10.0	2.0	17.1
	12.5	4.5	14.1
	15.0	7.0	11.6
	20.0	12.4	3.9
	25.0	17.9	0.0
Sample 2 Alfalfa (Natl. Alfalfa Co.) Heil Drier. Entering gases 1010° C., 1.5 min. Analyzed after 5 days' storage at room temperature. Original water 9.4%.	10.0	4.4	14.4
	12.5	4.5	12.3
	15.0	8.0	6.9
	20.0	13.9	00.0
	25.0	14.3	00.0
Sample 3 University alfalfa dried at 22° C. before fan and then dried in vacuum. Analyzed after 5 days' storage at room temperature. Original water 12.4%.	2.1	0.8	19.7
	5.0	0.9	19.1
	10.0	2.4	18.1
	15.0	7.2	12.6
	20.0	14.6	1.6
Sample 4 Alfalfa (Cerophyl Co.) Heil Drier.* Analyzed after 5 days' storage at room temperature. Original water 7.2%.	10.8	3.9	14.4
	12.5	4.9	13.4
	15.0	7.6	10.5
	20.0	15.7	0.4
	25.0	18.7	00.0
Sample 5 Oat grass (Cerophyl Co.) Heil Drier.* Analyzed after 5 days' storage at room temperature. Original water 10.1%.	10.0	3.5	16.3
	12.5	6.4	12.4
	15.0	10.2	5.9
	20.0	13.8	00.0
	25.0	14.8	00.0

* Carotene preservation complete (3 months' sealed storage) with 12 or 17 per cent total water.

Also used in these experiments was an alfalfa cut from a University field and dried before a fan at a temperature of 22–25° C. It then was dried in a vacuum to reduce the moisture content (sample 3).

Further studies were made on a dehydrated alfalfa (sample 4) and oat grass (Heil Drier, sample 5) in which the carotene was preserved completely when the products contained 12–17 per cent of water (alfalfa) and 15 per cent of water (oats) and were packed in flexo wax-covered receptacles and thus made airtight. These two materials gave most interesting data on the rate of oxygen disappearance and carbon dioxide production necessary for complete carotene preservation.

All of the products described above were packed in glass tubes 200 mm. long and 35 mm. in diameter and provided with stopcocks and airtight seals at both ends. They were held at room temperature (22–25° C.) for 5 days; then the contained gases were sampled quantitatively for oxygen and carbon dioxide. The data secured on the five materials are shown in table 1.

In all of the materials, the carbon dioxide production rate was not greatly different (7–10 per cent) where the total water content was 15 per cent. This measurement was made at the end of a 5-day storage period. With 20 per cent total water and a 5-day storage period, the rate of carbon dioxide production was practically doubled (12–15 per cent). The faster rate of carbon dioxide production and oxygen utilization at a higher water level than 15 per cent does not necessarily mean that a more efficient carotene preservation in an airtight receptacle will be secured by the use of the higher water level. In sample 4 (alfalfa), carotene was preserved completely with 12 and 17 per cent of water, respectively, when packed in airtight receptacles for 3 months. In the case of sample 5 (oats), 15 per cent of total water preserved the carotene completely under similar storage conditions. The destruction of carotene is a gradual process, with the concentration of oxygen a possible factor, but one not yet studied. The destruction of chlorophyll is associated with the high water levels and rapid carbon dioxide production. With 15 per cent of total water the original green color is retained much more effectively than when 20 per cent of total water is used. Since 12–15 per cent of total water in an airtight receptacle completely preserves the carotene, it appears that a commercially acceptable product in respect to both color and carotene readily can be prepared, provided an economical airtight receptacle becomes available.

To determine the influence of both time and water content on carbon dioxide production and oxygen consumption, a dehydrated alfalfa prepared by the Fulmer process and one prepared by the Heil dehydrator were put up in glass tubes with 10, 12.5, 15, 17.5, 20, and 25 per cent of total water. The gases in the tubes were analyzed at the end of 5, 10, 15, 20, 25, and 30 days. The tubes were held at room temperatures of 22–25° C. It also should be pointed out that when the total water content of both these alfalfas was 12.5 per cent, the carotene was preserved completely and the green color well retained when stored in waxed receptacles for 3 months at room temperature. With 10 per cent or less total water, the carotene loss under sealed conditions is variable and unpredictable. Dehydrated oats, rye, and alfalfa have shown carotene losses in 3 months of from 0.6 to 31 per cent. Most of the losses varied from 5–20 per cent. The results on the influence of time and water level on gas production and oxygen consumption are given in table 2.

TABLE 2

Rate of carbon dioxide production and oxygen consumption as influenced by level of water and time of storage

Storage time (days)	10% total water		12.5% total water		15% total water		17.5% total water		20% total water		25% total water	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
					Alfalfa* (Natl. Alfalfa Co.) Heil Drier, storage at 22–25° C.							
5	4.8	12.2	4.9	12.6	7.5	7.7	11.6	2.5	13.9	0.0	14.3	0.0
10	6.3	8.6	8.2	5.5	10.5	2.9	14.3	0.0	......	......	......	......
15	8.0	5.7	10.5	2.1	11.8	0.3	......	......	......	......	......	......
20	8.7	4.1	10.5	1.5	13.0	0.0	......	......	......	......	......	......
25	10.9	0.6	14.8	0.08	......	......	......	......	......	......	......	......
30	11.0	0.4	......	......	......	......	......	......	......	......	......	......
					Alfalfa* (Caple Bros.) Fulmer Drier, storage at 22–25° C.							
5	2.4	16.8	4.5	14.3	7.6	11.2	12.6	4.9	16.1	1.2	17.9	0.0
10	3.8	14.0	7.7	10.5	12.5	4.4	17.2	0.8	20.1	0.0	......	......
15	7.5	12.7	8.3	9.1	11.6	5.0	18.2	0.0	......	......	......	......
20	6.2	10.6	10.4	5.8	14.8	1.7	......	......	......	......	......	......
25	5.0	10.3	12.6	2.6	14.7	1.0	......	......	......	......	......	......
30	6.6	8.3	13.3	1.8	15.0	0.4	......	......	......	......	......	......

* Complete carotene preservation with 12.5% water and sealed storage at room temperature for 3 months.

It is clear from the data that carbon dioxide production and oxygen utilization definitely are related to both time of storage and the water levels. With 10 per cent of total water the rate of carbon dioxide production was distinctly reduced in the two materials examined. After 20 days of storage 4.1 per cent of oxygen still remained in the Heil dehydrated product and 10.6 per cent in the Fulmer dehydrated alfalfa. With 12.5 per cent of water and the same time of storage, the oxygen was reduced to 1.5 per cent in the first product and to 5.8 per cent in the second product. There was complete carotene preservation under sealed conditions with 12.5 per cent of total water in both materials on storage for 3 months at room temperature. Apparently reduced oxygen tension is an important factor in carotene preservation but to an extent as yet undetermined.

As the total water was increased, the reactions were more rapid. With 20 per cent of total water, the oxygen was consumed completely in 5 days with the Heil dehydrated alfalfa and 1.2 per cent remained in the Fulmer product. In 10 days the oxygen was consumed completely in the Fulmer material.

TABLE 3

Effect of temperature on carbon dioxide production and oxygen consumption in dehydrated alfalfa with 15% of total water (analysis at end of storage for 5 days)

Temperature	CO_2	O_2
	(%)	(%)
− 6° C.	1.4	19.7
+ 5° C.	2.0	18.2
+ 24° C.	8.0	10.5
+ 37° C.	19.4	0.8

In addition to the factors of time and water level on the extent of carbon dioxide production and oxygen consumption, the factor of temperature was investigated. Using the usual procedure, a dehydrated alfalfa was set up in glass tubes and stored for 5 days at varying temperatures with 15 per cent of total water. The results are given in table 3. As expected, the speed of the reaction was influenced markedly by the temperature as well as by the water level and time of storage. There was a fairly close agreement between the two alfalfas produced by the different drying processes in respect to rates of carbon dioxide production with variable water content. The longer exposure to a lower heat, as in the Fulmer process, still preserved the respiratory enzymes, with the rate of carbon dioxide production at a given water level only slightly lower.

Probably the differences in respiratory action of alfalfas dried by different processes are due only partially to the temperatures and time of drying. The stage of growth of the plant may be important.

SUMMARY

1. The rate of carbon dioxide production and oxygen consumption by dehydrated alfalfa and cereal grasses under sealed conditions was studied at moisture levels of 10–25 per cent. The rate of respiration increased as the level of moisture and the temperature were increased. The extent of carbon dioxide production and oxygen consumption was variable with time at a given moisture level.

2. The rate of carbon dioxide production in a dehydrated alfalfa with 10 per cent of total water is less than one-third the rate observed when the product contains 20 per cent of total water.

3. With 10 per cent or less of total water, the rate of carbon dioxide production and oxygen consumption generally is too slow to insure complete carotene preservation under sealed conditions. The carotene losses may vary from 5–30 per cent during storage at room temperatures of 22–25° C. for 3 months. With 12.5 per cent of total water under sealed conditions for 3 months, both products (alfalfa, Heil Drier, and alfalfa, Fulmer Drier) retained completely their carotene content as well as their green color.

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DEHYDRATED SWEET POTATOES—THEIR FEEDING VALUE AND DIGESTIBILITY

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Dehydrated sweet potatoes are being used more and more in the southern states as a carbohydrate feed for livestock. This has come about because of the increased acreage planted in sweet potatoes and the increase in the number of dehydrating plants. Although corn is the chief carbohydrate feed used, much of it has to be brought into the southern states since many areas cannot produce an adequate supply locally. The 1946 statistics (17) show that the 120,000 acres of sweet potatoes planted in Louisiana produced 10,824,000 bushels, of which approximately 13 per cent were dehydrated. At present there are 67 dehydrating plants in the state, 50 of which are used exclusively for dehydrating sweet potatoes (18). Most of the potatoes dehydrated are now sold as feed for livestock, and a smaller amount is being used as human food. The reverse was true during the war years.

LITERATURE REVIEW

Investigators in Tennessee (6, 11), Alabama (8, 9), Mississippi (3), South Carolina (7), and Oklahoma (4, 5) have made studies of the feeding value of dried sweet potato meal in finishing and fattening steer calves and hogs and as a substitute for corn in rations of beef cattle, mules, and hogs. In general, dried sweet potatoes were found to be worth approximately 90 to 95 per cent as much as corn for fattening beef cattle. Mules and hogs did not relish dried sweet potatoes but tolerated the meal to the best advantage when it replaced not more than 50 per cent of the corn in the ration.

Copeland (2) of Texas, in comparing dehydrated sweet potato meal and ground shelled corn for lactating cows, reported 0.79 lb. or 3 per cent more milk per cow daily in favor of corn. Thus, the dried sweet potato meal was 90 per cent as valuable as corn for milk production. Butter produced from cows on the sweet potato meal contained 37.98 I.U. of vitamin A per gram compared with 31.11 I.U. from butter produced by cows fed yellow corn.

Briggs *et al.* (1) of the Oklahoma Station recently reported on the digestibility of dried sweet potatoes by steers and lambs. Alfalfa or prairie hay and cottonseed meal were used as basal feeds, and tests on the digestibility of ground yellow corn also were run for comparative purposes. On the dry matter basis the average T.D.N. value of the dried sweet potatoes for steers was 86.05, as compared to 85.80 for the corn.

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EXPERIMENTAL

Feeding Trials

In 1943, sun-dried sweet potato meal made from a high-starch sweet potato variety, L–45, was fed in comparison with ground yellow corn meal to 16 Holstein and 4 Jersey milking cows. The double reversal experimental design (16) was used. This design compared two roughages and also two concentrate mixtures in the same feeding trial, utilizing the analysis of variance. The concentrate ration consisted of three parts of dehydrated sweet potato meal or corn meal and one part of cottonseed meal. One per cent each of salt, steamed bonemeal, and oyster shell flour were added. The mixtures were fed to the cows at the rate of 9 lb. per pound butterfat produced. In addition, each cow received approximately 30 lb. of corn silage and 17 lb. of either lespedeza or alyce clover hay daily. Daily 4 per cent fat-corrected milk yields and butterfat yields favored the yellow corn meal by 4.19 and 4.55 per cent, respectively. This trial indicated that the dehydrated sweet potato meal was equal to approximately 88 per cent corn.

In 1944 a second feeding trial was conducted with 16 Holsteins and 4 Jerseys, using a commercial dehydrated sweet potato meal of the Porto Rico variety. In this trial ground snapped corn (including cob and shuck), rather than corn meal, was compared to dehydrated sweet potatoes. Adjustment to equalize nutrient intake was made for the extra bulk caused by the cob and shuck by adding 20 per cent more of the ground snapped corn. The amount of grain mixture fed was established during the 2-week preliminary period using the recommended nutrient requirements as set forth by Morrison (12). Corn silage and lespedeza hay or alyce clover hay also were fed, as in the first trial. Milk production for the cows on the dehydrated sweet potatoes was 24.9 lb. per cow daily, as compared with 25 lb. for the cows on the ground corn. One pound of dehydrated sweet potato meal was equal to approximately 1.2 lb. of ground snapped corn (including cob and shuck). Vitamin A analyses of the butterfat from milk produced by cows on sweet potatoes were 19 per cent higher than on butterfat produced by cows on ground corn. The values were 43 I.U./g. and 35 I.U./g., respectively.

In 1946 a commercial dehydrated sweet potato meal produced by utilizing cull potatoes, peelings, bruised sections of potatoes, and similar material was used in a palatability study. This meal was darker in color than the previous products. It is now known that cut or spoiled potatoes turn dark upon dehydration. This product was not very palatable—many of the animals went off feed at various times. When a good quality product of dehydrated sweet potatoes produced from whole tubers was fed, normal amounts were eaten.

Digestion Trials

Four dairy steers weighing from 400 to 500 lb. each were used as experimental animals. The procedure used in the management of the animals and procuring the feed samples and excreta was the same as that reported in an earlier paper (13). In general, it included the use of at least a 10-day preliminary period and a 10-day collection period. The feces were collected in a canvas collection bag. A 1/50 aliquot was taken from each day's fecal output for each steer and stored in a refrigerator at 0° C. until completion of the collection period. Nitrogen determinations were made on aliquots of the fresh excreta, while dried excreta samples were used for the other analyses.

The basal feeds used were common lespedeza hay, alyce clover hay, or Kobe lespedeza hay and cottonseed meal. Digestion coefficients (13, 14, 15) were determined for these feeds prior to each sweet potato digestion trial.

Trial I was made on a 1945 commercial dehydrated sweet potato meal (Porto Rico variety), using common lespedeza hay as a basal feed. Four to five pounds of dehydrated sweet potato meal were fed to each steer and replaced an equivalent amount of hay on the digestible protein and T.D.N. basis in the basal ration. The amount of hay was adjusted so that the animals maintained their weights during the preliminary period, after which the digestion trial was begun. Trial II made use of alyce clover hay and a 1946 commercial dehydrated sweet potato meal; trial III, of alyce clover hay and a 1946 commercial dehydrated sweet potato meal made from cull and sectioned potatoes; trial IV, of Kobe lespedeza hay and cottonseed meal and a 1947 commercial dehydrated sweet potato meal. Table 1 presents the compositions of the basal feeds on the dry basis.

TABLE 1

Analyses of the basic feeds fed in the digestion trials (dry basis)

Trial	Feed	Dry matter	Crude protein	Crude fat	Crude fiber	N.F.E.
		(%)	(%)	(%)	(%)	(%)
I	Common lespedeza hay.	89.30	10.52	2.06	35.70	44.75
II	Alyce clover hay ..	92.14	13.22	1.45	38.62	40.09
III	Alyce clover hay . .	91.55	12.77	1.71	39.50	39.31
IV	Kobe lespedeza hay . .	87.80	11.53	1.94	39.92	40.77
	Cottonseed meal .	91.53	44.73	6.99	10.19	30.33

The compositions, average apparent coefficients of digestibility, and total digestible nutrients of the dehydrated sweet potatoes on the dry basis for the various trials are presented in table 2.

DISCUSSION

The apparent variations and non-digestibility of the protein, fat and fiber of the dehydrated sweet potatoes, as seen in table 2, might be explained by the small proportion of the total intake of these constituents in the ration. The apparent non-digestibility of the crude protein may be due to several factors, namely: (a) heating of the protein during the drying process, which could tend to make it less digestible, (b) the presence of non-protein nitrogen compounds that are not digestible, or (c) an apparent "depression of digestibility" as observed by Holdaway *et al.* (10) when a high carbohydrate feed such as apple pomace was added to a basal ration low in protein. In digestion trials with steers, Briggs *et al.* (1) obtained an apparent digestibility of crude protein in their sun-dried sweet potatoes amounting to 42.7 ± 8.36 per cent and 59.0 ± 7.3 per cent with prairie hay and cottonseed meal, and alfalfa, respectively. Their sun-dried sweet potatoes were higher in composition of crude protein, 7.15 per cent, than the values of 4.17 to 5.39 per cent reported in this paper. However, these investigators also experienced considerable difficulty in obtaining the apparent digestibility of the fat and fiber from their sun-dried sweet potatoes; the apparent digestibility of crude fiber seemed to be enhanced by the basic feed of alfalfa hay. Their T.D.N. values for sweet potatoes when fed to steers were 81.7 with prairie hay and cottonseed meal as the basal feed and 91.4 with alfalfa hay as the basal feed.

TABLE 2

The composition, average apparent digestion coffieoients, and total digestible nutrients of commercial dehydrated sweet potatoes (dry basis)

Trial	Year of manufacture	Dry matter	Protein	Fat	Fiber	N.F.E.	
		(%)	(%)	(%)	(%)	(%)	
I	1945	92.70	5.39	0.91	4.21	86.28	
II	1946	92.96	5.04	1.47	1.67	88.63	
III	1946†	90.40	5.04	0.55	4.78	85.99	
IV	1947	85.35	4.17	1.15	4.46	84.88	
			Average apparent digestion coefficients				T.D.N.
I	1945		16.71			94.46	81.06
II	1946		−24.74	49.07		84.53	76.38
III	1946*†		−52.36			83.47	71.78
IV	1947		−40.29	94.17		88.23	79.31

* Analyses based on three steers only; product, made from cull and sectioned potatoes, was slightly unpalatable, one of the steers refusing to eat his quota.
† Poor quality.

In this study the coefficients of digestibility for the N.F.E. were consistent. As this constituent made up over 84 per cent of the dry matter in the dehydrated sweet potato, the apparent lack of digestibility of the other constituents had but small effect on the T.D.N. content. The low content of protein precludes this product from being an important source of this nutrient. The T.D.N. values for commercial dehydrated sweet potatoes ranged from 76 to 81 per cent on the dry basis; the poor quality commercial dehydrated sweet potatoes made from culled potatoes, peelings, and bruised sections of potatoes contained 71 per cent T.D.N.

SUMMARY

1. Dehydrated sweet potatoes made from whole tubers were palatable, while a poor quality product made from culled, bruised, and sectioned potatoes was slightly unpalatable.
2. Feeding trials have shown that dehydrated sweet potatoes have approximately 88 per cent the value of yellow corn meal, and are approximately 17 per cent more valuable than ground snapped corn, including cob and shuck, for milk production.
3. Digestion trials have shown that good quality dehydrated sweet potatoes contained from 76 to 81 per cent T.D.N., while a poor quality product contained 71 per cent T.D.N. on the dry basis.
4. Dehydrated sweet potatoes are of value in the dairy ration, not only because of the high digestibility of their carbohydrate, but also because of their high carotene content, which increases the vitamin A value of the butterfat.

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GRASS AND LEGUME SILAGE STUDIES USING 2-QUART GLASS JARS AS MINIATURE SILOS[1, 2]

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The present emphasis on grassland farming and the recognition of grass silage as a practical feed have raised many questions regarding methods of preservation, losses that occur in the silo and investigational technics for silage studies. Workers at the Pennsylvania Station (12) have made extensive studies of bacteriological and chemical changes in grass silage put up with various preservatives. The silages used in these investigations were stored in small wooden silos 5 feet in diameter and 20 feet high. While these small silos adapted themselves much better to sampling than would larger commercial structures, the job of procuring representative samples at different intervals after ensiling presented a difficult problem.

Studies have been made using glass jars and even milk bottles as miniature silos (2, 4, 9), and results have shown that silage preserved in this manner was similar in composition and appearance to that stored in conventional silos.

To study further the practicability of using 2-quart glass jars as silos, and to study fermentation changes under different treatments, an experiment was designed in the spring of 1946.

EXPERIMENTAL PROCEDURE

The silages used in this experiment were: A, alfalfa; B, brome grass-Ladino clover; C, orchard grass-Ladino clover. Each was studied under the following treatments:

1—Unwilted, no preservative.
2—Unwilted, plus 60 lb. of molasses per ton of green material.
3—Unwilted, plus 100 lb. of hominy feed per ton of green material.
4—Unwilted, plus 300 lb. of wheat straw per ton of green material.
5—Wilted, no preservative.
6—Wilted, plus 60 lb. of molasses per ton of green material.
7—Wilted, plus 100 lb. of hominy feed per ton of green material.

The silages hereinafter will be designated according to the above numbers and letters. For example, A-1 is alfalfa, unwilted, with no preservative added.

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The material to be ensiled was chopped with a Fox ensilage cutter set to cut 0.25-inch lengths. In the case of treatments 1 through 4, the material was prepared with the proper amount of preservative and ensiled as quickly as possible after cutting. In treatments 5 through 7, the crop was wilted to approximately 65 per cent moisture before ensiling. Each treatment was replicated ten times in glass jars of 2-quart capacity. It was estimated that one such jar would provide an adequate sample for each analysis, the analyses to be made at intervals of 1, 7, 14, 30, 60, and 170 days from the ensiling date. The silage was packed thoroughly; the jars were sealed with rubber jar rings and paraffin wax and stored at 60 to 70° F.

Samples were examined at the intervals named above, and the following measurements and observations were made:

(a) Odor and apparent quality of the ensiled material.
(b) pH of the silage extract. This determination was made with a Beckman pH meter.
(c) Dry matter. Moisture was removed from samples by oven-drying at 95° C.
(d) Sugar. The silage extract was prepared and treated according to the method used by McAuliffe (6), and sugar determinations were made using the method of Stiles *et al.* (11).
(e) Nitrogen. Analyses were made on samples of all the silages at intervals of 1 and 60 days following ensiling.
(f) Carotene. The silages in B and C groups were analyzed for carotene content at intervals of 1, 7, 14, and 30 days after the ensiling date. The method of Moore and Ely (7) was used in these determinations.

Group A was ensiled about 2 months later than the other two; hence, in most of the data reported here, it will be considered separately from groups B and C. Lactic acid determinations were made on the alfalfa silages, samples being taken at intervals of 1, 3, 5, 7, 10, 14, 30, 60, and 110 days from the ensiling date. The lactic acid method used was a modification of that of Barker and Summerson (1).

RESULTS AND DISCUSSION

Table 1 shows the dry matter content of the silages studied in this experiment, and table 2 gives an analysis of variance of these dry matter values. In this analysis, differences among treatments were highly significant. This was expected, since in three cases the silages were wilted. Although differences among the means of the various sampling intervals were noticeable, there was no consistent increase or decrease in moisture content from the date of ensiling to 60 days later. This is not surprising, as there was no chance for a loss of moisture due to seepage, which might have occurred in a conventional silo.

TABLE 1

Per cent dry matter of 14 different silages as estimated at various sampling intervals

Group	Interval from ensiling date (Days)	Treatments							
		1	2	3	4	5	6	7	Mean
B	1	29.59	30.53	31.63	38.97	40.86	42.31	43.27	36.74
	7	28.77	29.76	30.44	36.92	40.19	40.98	41.51	35.51
	14	29.22	29.15	30.84	36.62	39.29	38.20	41.80	35.02
	30	28.27	29.24	30.48	38.48	39.69	41.26	42.83	35.75
	60	29.64	30.09	29.53	37.15	39.99	41.04	41.93	35.62
	Mean	29.10	29.75	30.58	37.63	40.00	40.76	42.27	35.73
C	1	23.60	23.37	24.44	30.00	31.95	32.70	34.00	28.58
	7	24.49	25.04	26.23	31.47	33.16	32.27	34.06	29.53
	14	22.47	23.72	25.89	29.84	32.43	31.36	33.71	28.49
	30	21.01	21.30	24.49	29.72	31.12	31.57	33.51	27.53
	60	22.37	23.52	24.18	29.56	31.31	31.54	33.04	27.93
	Mean	22.79	23.39	25.05	30.12	31.99	31.89	33.66	28.41

The most striking fact revealed by this analysis is the small standard deviation. In group B the mean and standard deviation were 35.73 ± 0.677, the coefficient of variation being 1.88 per cent. In group C, corresponding values were 28.41 ± 0.608 and 2.14 per cent, respectively. Analysis of B

TABLE 2

*Analysis of variance of dry matter content of 14 different silages at various sampling intervals**

Source of variance	Degrees of freedom	Sums of squares	Mean squares
	B		
Total	34	1002.61	
Treatment	6	960.47	163.41†
Interval	4	11.06	2.77†
Error	24	11.08	0.46

Mean = 35.73.
Standard deviation = 0.677.
Coefficient of variation = 1.88%.

Source of variance	Degrees of freedom	Sums of squares	Mean squares
	C		
Total	34	642.85	
Treatment	6	617.93	102.99†
Interval	4	16.05	4.01†
Error	24	8.87	0.37

Mean = 28.41.
Standard deviation = 0.608.
Coefficient of variation = 2.14%.

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* Treatment 4 was omitted from the analysis in order that uniform comparisons could be made of the wilted and unwilted silages.

† Highly significant.

and C together, and including treatment 4, gives a general mean and standard deviation of 32.07 ± 1.00. The coefficient of variation in this case is 3.12 per cent. Such small standard deviations indicate that sampling variation, normally high in silage experiments, can be kept to a negligible minimum in carefully controlled experiments using fruit jars as miniature silos. It is suggested by Snedecor (10) that a 5 per cent coefficient of variation is an excellent value in a well controlled field crop experiment.

It generally is recognized that a measure of the pH of a silage provides a very good indication of silage quality. In this experiment, therefore, a careful record was made of pH values at various intervals. Table 3 shows the pH values of all the silages studied in this experiment. The analysis of variance of groups B and C is found in table 4.

TABLE 3

pH values of 21 silages at different intervals

Group	Interval from ensiling date (Days)	Treatments							
		1	2	3	4	5	6	7	Mean
B	1	4.70	4.53	4.59	4.88	4.98	4.87	4.91	4.78
	7	4.02	3.92	3.93	4.37	4.18	4.15	4.12	4.10
	14	4.06	4.03	4.05	4.50	4.39	4.19	4.16	4.20
	30	4.09	4.01	3.99	4.31	4.09	4.09	4.09	4.10
	60	3.98	3.81	3.99	4.18	4.02	4.01	3.99	4.00
	170	3.99	3.95	4.00	4.33	4.13	4.13	4.08	4.09
	Mean	4.14	4.04	4.09	4.43	4.30	4.24	4.23	4.17
C	1	4.92	4.78	4.72	5.01	5.19	4.94	5.02	4.94
	7	4.20	4.03	4.05	4.39	4.50	4.45	4.45	4.30
	14	4.10	4.09	4.09	4.32	4.44	4.30	4.39	4.25
	30	4.08	3.98	3.93	4.21	4.27	4.18	4.14	4.11
	60	4.07	4.00	3.97	4.18	4.25	4.16	4.15	4.11
	170	4.21	4.01	4.08	4.18	4.19	4.11	4.12	4.13
	Mean	4.26	4.15	4.14	4.38	4.47	4.36	4.38	4.29
A	1	5.04	4.71	5.98	5.68	5.41	5.22	5.39	5.35
	3	4.68	*	4.42	4.87	4.87	4.53	4.80	4.70
	5	4.66	4.28	4.39	4.78	4.78	4.32	4.67	4.55
	7	4.61	*	4.36	4.76	4.78	4.28	4.59	4.56
	10	4.55	*	4.29	4.68	4.78	4.28	4.58	4.53
	14	4.51	4.10	4.20	4.59	4.76	4.19	4.52	4.41
	30	4.48	*	4.25	4.66	4.70	4.19	4.43	4.45
	60	5.19	*	4.18	4.73	4.63	4.21	4.41	4.56
	110	4.58	*	4.12	4.79	4.62	4.08	4.30	4.42
	Mean	4.70	4.36	4.47	4.84	4.81	4.37	4.63	4.62

* Missing values due to loss of samples in storage.

The most marked differences to occur were among intervals. This was to be expected, since it is established that in the fermentation of most silages some time is required for maximum acidity to develop. The difference between groups also was highly significant. The lower moisture content of the silages in group B may have had a bearing in this connection.

Among treatments within both the wilted and unwilted groups, the differences were highly significant. In two cases the molasses-treated silages had the lowest pH values and in two cases the hominy-treated silages were lowest; but in all cases the untreated silages were high. The difference between wilted and unwilted silages, irrespective of variation among treatments, was highly significant, the mean pH of the former being 4.14 and that of the latter 4.33. Corresponding values for silages tested after 60 days from the ensiling date were 4.01 and 4.11, respectively. The discrepancy between these two pairs of values may be explained by the highly significant interaction between interval variance and the variance between unwilted and wilted silages. This discrepancy indicates that the fermentation in the wilted silages proceeded at a somewhat slower rate than in the unwilted group. Even after 170 days, some of the silages which had been wilted still were increasing in acidity. This characteristic of slow fermentation was most noticeable in the alfalfa group (see table 3, group A).

TABLE 4

*Analysis of variance of pH values**

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Total	71	7.0079	
Treatments:			
Within unwilted	2	0.0767	0.0384†
Within wilted	2	0.0590	0.0295†
Between unwilted & wilted	1	0.6574	0.6574†
Groups	1	0.2616	0.2616†
Intervals	5	5.6152	1.1230†
Int. × group	5	0.0711	0.0142†
Int. × (between unw. & w.)	5	0.1124	0.0225†
Int. × within unw.	10	0.0234	0.0023
Int. × within w.	10	0.0236	0.0024
Group × (between unw. & w.)	1	0.0139	0.0139
Group × within unw.	2	0.0093	0.0047
Group × within w.	2	0.0052	0.0026
Int. × group × (between unw. & w.)	5	0.0470	0.0094
Int. × group × within unw.	10	0.0154	0.0015
Int. × group × within w.	10	0.0167	0.0017

* Analysis made only on groups B and C, and treatment 4 was omitted.
† Significant at the 1% level.

Standard deviation = 0.075.
General mean = 4.233 ± 0.075.
Coefficient of variation = 1.77%.

The general mean and standard deviation for the pH values of groups B and C were 4.233 ± 0.075, the coefficient of variation being 1.77 per cent. This low coefficient of variation corroborates a previous observation that sampling variation in this experiment has been kept to a minimum.

Examination of the data reported on extensive grass silage experiments at the Pennsylvania Station (6) revealed a pronounced inverse relationship between the pH value and sugar content of grass silage. A rather marked direct correlation also was found between the reducing substances and lactic acid content of the grass silages studied in these trials. Appreciable quantities of both reducing substances and lactic acid were associated with high-quality grass silage. Therefore, it was deemed advisable to make sugar determinations at various intervals on the silages reported herein. Table 5 contains a summary of the values derived from these determinations, and table 6 shows the analysis of variance.

The analysis in table 6 shows a highly significant variation among treatments within both the wilted and unwilted silages. In all cases except one, the sugar content of the molasses-treated silage was highest; that of the hominy-treated silage was intermediate, and those of silages receiving no preservative were lowest. There also were marked differences between the wilted and unwilted silages, the wilted silages containing 40 per cent and 33 per cent more sugar than the unwilted samples in groups B and C, respectively. The difference between intervals also proved to be highly significant. The table likewise shows a noticeable interaction between groups and treatments, within the wilted silages. Although this interaction is barely significant, it indicates that the effects of treatment on wilted silage in group B were different from those in group C. In the latter group, treatment 5 showed to a better advantage than treatment 7. This interaction further suggests that the moisture content of the wilted silage in C is more conducive to desirable fermentation than that in B (see table 1).

TABLE 5

Glucose content of 14 silages at various sampling intervals
(Milligrams per gram of silage—dry basis)

Groups	Interval from ensiling date (Days)	Treatments							
		1	2	3	4	5	6	7	Mean
B	1	3.81	9.14	3.63	1.73	1.63	11.97	9.96	5.98
	7	12.99	22.15	7.68	8.24	16.43	16.19	15.07	14.11
	14	4.47	9.31	5.20	7.87	11.48	15.26	11.85	9.35
	30	5.64	10.79	6.64	8.69	12.28	15.68	14.27	10.57
	60	6.32	18.59	8.27	9.81	12.90	15.65	13.96	12.21
	170	6.29	14.55	11.87	10.82	11.90	14.55	14.08	12.01
	Mean	6.59	14.09	7.22	7.86	11.10	14.88	13.20	.
C	1	2.37	11.37	2.00	1.36	1.88	5.14	3.12	3.89
	7	2.76	14.02	5.11	3.62	4.78	14.24	4.55	7.01
	14	2.24	7.57	5.27	3.55	5.44	9.75	4.13	5.42
	30	1.62	7.82	5.67	5.61	6.06	14.08	3.48	6.33
	60	4.96	7.44	8.42	6.11	8.38	15.21	6.89	8.20
	170	2.50	5.73	7.31	5.18	7.91	14.64	9.30	7.51
	Mean	2.74	8.99	5.63	4.24	5.74	12.18	5.25	. .

Silage samples from group A were analyzed for lactic acid content at various intervals. The results of this study are recorded in table 7. Analysis of variance illustrates that differences among treatments were highly significant. Although the means of the unwilted silages are high in this comparison, the wilted samples had undergone the most consistent increase in lactic acid content and the quality of the wilted silages apparently was superior to that of the unwilted silages in later stages of the experiment. The silage prepared with 300 lb. of wheat straw per ton developed decidedly less lactic acid than those under any other treatment. Differences among intervals were large but quite variable.

TABLE 6

*Analysis of variance of glucose content of 14 different silages at various sampling intervals**

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Total	71	1665.893	
Treatments:			
Within unwilted	2	306.231	153.116†
Within wilted	2	181.148	90.574†
Between unwilted & wilted	1	146.120	146.120†
Groups	1	352.407	352.407†
Intervals	5	278.046	55.609†
Int. × group	5	42.490	8.498
Int. × (between unw. & w.)	5	47.391	9.478
Int. × within unw.	10	81.660	8.166
Int. × within w.	10	26.636	2.636
Group × (between unw. & w.)	1	15.097	15.097
Group × within unw.	2	19.005	8.503
Group × within w.	2	41.294	20.647‡
Int. × group × (between unw. & w.)	5	34.093	6.819
Int. × group × within unw.	10	50.240	5.024
Int. × group × within w.	10	44.034	4.403

* Analysis does not include treatment 4.
† Significant at the 1% level.
‡ Significant at the 5% level.

Standard deviation = 2.588.
General mean = 8.967 ± 2.588.

Samples of the silages in all three groups were taken for crude protein analysis at intervals of 1 and 60 days after ensiling. The protein content of the different silages is shown in table 8. The analysis of variance shows a highly significant treatment effect, which is due to the lower protein values in treatment 4. When this treatment is omitted from the analysis, treatment effects are not significant.

The most pronounced effects occurred between groups, because of the much higher protein content of the silage in A, the alfalfa group. There was a highly significant difference between intervals in this analysis. In every case except two, the protein content of silage sampled on the first day was higher than that sampled 60 days after ensiling. The average decrease from 1 to 60 days was 4.90 per cent. It is difficult to explain this loss in nitrogen but probably it was due to ammonia, formed during fermentation, which was lost during sampling.

There was a noticeable interaction between treatment and groups, but this effect was not significant when the straw-treated silages were omitted, and the interaction probably has no practical importance.

A comparison was made of treatments 1 and 3, which were unwilted, with 5 and 7, which were the corresponding treatments in the wilted group.

TABLE 7

Micrograms of lactic acid per gram of silage in group A (Dry basis)

Interval from ensiling date (Days)	Treatments*						
	1	3	4	5	6	7	Mean
1	13.38	2.71	7.23	14.44	19.02	7.66	10.74
3	44.75	51.41	29.01	27.79	33.42	29.80	36.03
5	34.54	38.55	24.85	27.29	37.54	30.24	32.17
7	37.53	42.65	26.74	29.30	40.64	34.48	35.22
10	27.08	29.52	19.48	28.38	39.60	30.00	29.01
14	65.16	54.88	37.17	36.41	43.73	39.72	46.18
30	60.76	57.09	33.69	39.14	44.14	38.71	45.59
60	37.68	58.67	32.53	39.35	42.08	39.72	41.67
110	41.38	49.21	22.21	34.32	45.59	36.70	38.24
Mean	40.25	42.74	25.88	30.71	38.42	31.89	34.98

Analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Total	53	8878.57	
Treatment	5	1893.97	378.79†
Interval	8	5553.59	694.20†
Error	40	1431.01	35.78

* Treatment 2 has been omitted because of the loss of a number of samples in storage.

† Significant at the 1% level.

Standard deviation = 5.98.

Mean = 34.98 ± 5.98.

The wilted silage contained an average of 2.26 per cent less protein than the unwilted silage. Table 9 shows the effect of the seven treatments on carotene content of the ensiled crops.

Excluding treatment 4, in which carotene values were low chiefly because of the straw content, the wilted silages contained 30 per cent and 37 per cent less carotene than the unwilted crops in groups B and C, respectively. Although the carotene values reported in table 9 are quite variable, there was an apparent increase in carotene content of the silages from the date of ensiling to 30 days later. In only two cases were decreases noted.

These results confirm those obtained at other stations (3, 5, 8), where it was found that the carotene content in silage was higher than in the original green forage.

The quantities of silage used in these studies precluded the possibility of making palatability tests or running feeding trials; but a careful record was kept of the odor and apparent quality of the various silages at different intervals. In general those silages with pH values of less than 4.2 were of desirable quality and well preserved. The unwilted silages in groups A and C, irrespective of treatment, were, in the later stages of fermentation, inferior to the wilted silages in the same groups. In group B this was not true, probably due to the relatively low moisture content of the unwilted silages. The only silage which was considered of poor quality and had a

TABLE 8

Percentage of crude protein (dry matter basis) in 18 silages at 1 day and 60 days after ensiling

Groups	Interval from ensiling date (Days)	Treatments*						
		1	3	4	5	6	7	Mean
A	1	17.06	16.19	12.50	15.94	16.63	16.19	15.75
	60	16.25	15.88	11.56	16.00	15.50	16.38	15.26
	Mean	16.66	16.04	12.03	15.97	16.07	16.29	15.51
B	1	11.94	11.75	8.25	10.19	11.19	10.63	10.66
	60	10.44	10.94	8.00	9.50	10.19	10.06	9.86
	Mean	11.19	11.35	8.13	9.85	10.69	10.35	10.26
C	1	11.00	11.50	9.13	11.38	10.81	11.63	19.91
	60	10.00	10.81	8.50	11.31	10.69	11.00	10.39
	Mean	10.50	11.16	8.82	11.35	10.75	11.32	10.65

Analysis of variance

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Total	35	263.0660	
Treatments	5	45.1541	9.0308†
Groups	2	205.3368	102.6684†
Intervals	1	3.3003	3.3003†
Treat. × group	10	7.5695	0.7570†
Treat. × int.	5	0.7193	0.1439
Group × int.	2	0.1777	0.0889
Treat. × group × int.	10	0.8083	0.0808

* Treatment 2 omitted because of missing data.

Mean decrease in protein from 0 to 60 days: A = 3.11%; B = 7.50%; C = 4.77%.

† Significant at the 1% level.

Standard deviation = 0.586.
General mean = 12.14 ± 0.586.
Coefficient of variation = 4.83%.

TABLE 9

Carotene content of grass silage under seven different treatments
(Expressed as micrograms of carotene, per gram of silage—dry basis)

Groups	Days after ensiling	Treatments							
		1	2	3	4	5	6	7	Mean
B	1	102.1	105.9	117.1	57.1	56.3	80.5	81.6	85.8
	7	*	*	75.2	42.5	78.8	79.7	*	69.1
	14	125.8	116.6	111.8	43.1	80.0	73.1	71.7	88.9
	30	135.4	129.4	137.0	67.8	98.5	109.2	115.7	113.3
	Mean	121.1	117.3	110.3	52.6	78.4	85.6	89.7	
C	1	*	*	*	*	114.4	85.9	75.5	91.9
	7	*	*	123.0	68.4	104.4	130.4	83.5	101.9
	14	195.3	179.1	148.1	127.8	129.1	133.3	90.0	143.2
	30	140.4	203.1	192.4	118.2	105.7	100.3	95.1	136.5
	Mean	167.9	191.1	154.5	104.8	113.4	112.5	86.0	

* Missing values due to loss of samples.

decidedly unpleasant odor was that prepared under treatment 1 of group A. Some of the samples, which had a moisture content of only 60 per cent, were of good quality but appeared quite dry and probably would not be the most palatable to cows. Even after 170 days, fermentation had not ceased in some of the silages prepared from wilted materials.

SUMMARY

1. On the basis of the results obtained in these experiments, it appears that the use of 2-quart glass jars as miniature silos offers a convenient technic for studies in silage preservation.

2. Desirable fermentation was obtained in all cases except the unwilted, untreated alfalfa silage. It should be noted that in the silages of groups B and C there was very little clover, and the crops were in full bloom.

3. The development of acidity was more rapid in the unwilted than in the wilted silages, and even after 170 days some of the wilted silages were still increasing in acidity. This characteristic was especially noticeable in the alfalfa group.

4. Using pH values as a measure of desirable fermentation, 60 lb. of molasses and 100 lb. of hominy feed, respectively, were equally effective as preservatives under the conditions of these experiments. Silages with straw added, and that wilted plus no preservative, were well preserved and of good quality, but were somewhat inferior to those in which preservatives were used.

5. There was no destruction of carotène in any of the silages up to 30 days following ensiling, an actual increase being noted in most cases.

6. The mean sugar content of the wilted silages was 40 and 33 per cent higher than for the unwilted silages in groups B and C, respectively.

7. The crude protein content of samples analyzed 60 days after ensiling was 4.90 per cent less than that of silage 1 day old. There also was a slight loss of crude protein, attributable to wilting.

8. Analysis of variance of dry matter, nitrogen, and pH values of the various silages studied here revealed a coefficient of variation of less than 5 per cent in all cases and less than 2 per cent in some instances. This extremely low variability indicates that very accurate sampling can be accomplished by using glass jars as miniature silos. Another important advantage of the miniature silo technic is that samples may be taken at any interval desired following the ensiling date.

9. Evidently the fermentation changes which occur in 2-quart glass jar silos are similar to those which occur in commercial silos.

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THE PLACENTAL TRANSFER AND COLOSTRAL STORAGE OF VITAMIN D IN THE BOVINE[1]

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The possible importance of prenatal and neonatal environment on the development and subsequent performance of dairy animals has been recognized for many years, but there is little experimental evidence bearing on this view. Recently several investigators (2, 15, 16, 23) have shown that the level of vitamin A in the newborn calf and in the colostrum is related directly to the vitamin A content of the prepartal diet. These investigations and the reports of rickets occurring in very young calves (1, 5) raised the question of the value of supplementing the prepartum diet with vitamin D.

The objective of the study reported herein was to determine the effect of prepartal diet and of season, respectively, on the level of vitamin D in the blood plasma of the pregnant cow, in the blood plasma and the liver of the newborn calf, and in the colostrum of the dam.

EXPERIMENTAL

Animals. Four groups of Holstein cows in the Cornell University herd were used in this experiment. The cows were allotted to an experimental group according to age, condition, health, ancestry, and previous performance. Two groups of four cows each were started on experiment during March, 1946, and one group of four cows and one group of five cows during August, 1946, to represent the latter part of the winter feeding period and the latter part of the pasture season, respectively. At each season one group of cows served as controls and the other group received a supplement of vitamin D. Each cow in the supplemented group was given 100,000 I.U. of vitamin D in the form of irradiated dry yeast[2] once a day for 8 weeks prepartum. The cows started on experiment in March were kept in the barn except for approximately 1 hour daily, when they were turned out for exercise in a cindered lot. The cows started on experiment in August were on pasture except for two periods of approximately 3 hours daily, when they were brought to the barn for feeding. The following summary gives the feeds fed and their carotene and vitamin D contents. Feed intakes were not recorded.

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[1] This paper is part of a thesis presented by H. D. Eaton to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Capsules manufactured to contain 100,000 I.U. of vitamin D by Standard Brands, Inc., New York, N. Y.

Feed	*Description*	*Carotene ug./lb.*	*Vitamin D I.U./lb.*
	Spring, 1946		
Timothy hay	U. S. no. 2, first cutting at full bloom to late maturity, and field cured.	2,419	658
Corn silage	Fairly well-eared silage, early dent stage, containing 28 per cent dry matter on an air-dry basis.	6,532	70
Grain	A fitting ration consisting largely of cereal grains and containing 12 per cent total protein.	....	....
	Fall, 1946		
Timothy hay	U. S. no. 2, first cutting at late maturity, and field cured.	3,688	909
Corn silage	Well-eared corn silage, early to middle dent stage, containing 24 per cent dry matter on an air-dry basis.	3,289	68
Grain	A fitting ration consisting largely of cereal grains and containing 12 per cent total protein.	....	....
Pasture	Predominately bluegrass.		

Samples. Maternal blood samples, taken at 8 weeks prepartum, before vitamin D supplementation, and at 2 weeks prepartum, were obtained between 7 and 8 a.m. from the jugular vein. Saturated aqueous sodium citrate was used as an anticoagulant. The sample was cooled to approximately 40° F., and centrifuged at 2,500 r.p.m. for 20 minutes. The plasma was siphoned off into 100-ml. sample bottles and held at approximately 0° F. until assayed for vitamin D.

Calves were slaughtered within 3 hours after birth and before they had nursed. Blood plasma was treated as described above. Livers from calves born in the spring of 1946 were cooled to 38° F., cut into small pieces, placed on an enamel tray in an oven which had a constant circulation of air provided by an electric fan, and held at 60° C. for 24 hours. At the end of this period, they were removed from the oven, allowed to reach a constant weight at room temperature, ground in a Wiley mill, and held at 38° F. until assayed for vitamin D. After chilling to 38° F., livers from calves born in the fall of 1946 were frozen at 0° F., cut into small pieces, placed on small wire baskets in Pyrex glass desiccators containing phosphoric anhydride, and dried for 4 days under a partial vacuum. After drying, the livers were finely ground in a meat grinder and held at approximately 0° F. until assayed for vitamin D. The livers from calves born in the fall were freeze-dried because it was thought that some vitamin D might have been destroyed in the oven-drying procedure originally used. Limited data (10), however, do not indicate an appreciable difference in the two methods of drying.

The cows were milked twice daily and the colostrum was collected from the first six milkings postpartum. The colostrum was held at 34° F. until all six milkings were obtained. The entire yield of colostrum was heated to

100° F., separated with a centrifugal separator, and an aliquot of the resulting cream was held at 38° F. until assayed for vitamin D.

Analyses. Vitamin D determinations[3] were made according to the rat assay method essentially as set forth in the U. S. Pharmacopoeia XII (19). The approximate level of each type of sample to be fed was determined by preliminary assays. Photographs were made of the stained bones as a matter of record.[4] The vitamin D content was calculated by establishing an equation for a curve of response essentially as outlined by Coward (3). In the case of the colostrum, it was assumed that the vitamin D was contained only in the fat portion. Therefore, the fat content of the colostrum and colostrum cream was determined by means of the standard Babcock procedure.

The data were treated statistically by analysis of variance to determine significance as described by Snedecor (14). Due to the limited number of observations, only those differences significant at the 1 per cent level are reported as statistically significant in the results.

RESULTS

Maternal blood plasma vitamin D values are listed in table 1. Vitamin D

TABLE 1

Effect of prepartum vitamin D supplementation on maternal plasma vitamin D

March–April			Aug.–Sept.		
No. of cow	Plasma vitamin D		No. of cow	Plasma vitamin D	
	8 weeks* prepartum	2 weeks prepartum		8 weeks* prepartum	2 weeks prepartum
	(*I.U./100 ml.*)			(*I.U./100 ml.*)	
		Controls			
101	72	162	121	466	
107			129	351	163
111	85	171	131	193	324
Mean ± S.E.	79 ± 6	167 ± 4		337 ± 79	244 ± 85
		Vitamin D supplemented			
104†	115	439	122	278	475
108	81	160	126	337	306
112	112	247	128	425	437
Mean ± S.E.	103 ± 11	282 ± 82	.	347 ± 43	406 ± 51
		All cows			
Mean ± S.E.	93 ± 9	......	..	342 ± 40	

* Before supplementation with vitamin D.

† This cow was fed 200,000 I. U. daily of vitamin D 8 weeks prepartum.

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[3] These assays were made by the Laboratory of Industrial Hygiene, Inc., 76 Madison Ave., New York, N. Y., under the direction of Miss Grace McGuire.

[4] Complete reports are on file in the Department of Animal Husbandry, Cornell University.

increased to a higher level in the supplemented cows than in the controls, although this difference was not statistically significant. There was a statistical difference between seasons, the level of vitamin D being on an average two to three times higher during the fall than during the spring.

The vitamin D content of plasma of newborn calves is presented in table 2. Calves from supplemented dams tended to be higher in plasma vitamin D in the spring and slightly lower in the fall as compared to calves from control dams. There is an indication of a seasonal trend. Calves from both groups in the fall had higher plasma vitamin D levels than calves born in the spring. Neither the treatment difference nor the seasonal difference was statistically significant.

TABLE 2

Effect of prepartum vitamin D supplementation on plasma vitamin D in the newborn calf

April–May		Sept.–Oct.	
No. of dam	Vitamin D in plasma (*I.U./100 ml.*)	No. of dam	Vitamin D in plasma (*I.U./100 ml.*)
Controls			
101	32	121	131
107	55	129	126
111	52	131	75
Mean ± S.E.	46 ± 7	. .	111 ± 18
Dams vitamin D supplemented			
104*	111	122	95
108	73	126	91
112	42	128	91
Mean ± S.E.	75 ± 20	.. ....	92 ± 1

* Dam fed 200,000 I.U. daily of vitamin D 8 weeks prepartum.

No consistent differences were found between the vitamin D content of the livers of the newborn calves from the supplemented dams and from the control dams, as shown in table 3. On the other hand, there was an apparent seasonal trend. Both groups of calves born in the fall had higher levels of liver vitamin D than the calves born in the spring. This difference was not statistically significant.

The vitamin D values for colostrum are presented in table 4. The average vitamin D content of the colostrum was consistently higher in the supplemented groups than in the control groups. This difference was not statistically significant when comparing treatments at either season alone; however, when both seasons together were compared, this difference was statistically significant. There was a seasonal trend in colostrum vitamin D levels. The values during the fall were materially higher than those in the

TABLE 3

Effect of prepartum vitamin D supplementation on the vitamin D content of the liver in the newborn calf

No. of dam	Birth weight	Liver		
		Fresh weight	I.U./g.	Total I.U. in liver
	(lb.)	*(g.)*		
		April–May—Controls		
101	97	850	0.08	68
107	86	846	0.14	118
111	107	1064	0.06	64
Mean ± S.E.	... ..	. ..	0.09 ± 0.02	83 ± 17
		April–May—Dams vitamin D supplemented		
104[*]	81	648	0.20	130
108	86	655	0.07	46
112	89	667	0.06	40
Mean ± S.E.	.. ...	....	0.11 ± 0.05	72 ± 29
		Sept.–Oct.—Controls		
121	96	1115	0.30	334
129	100	862	0.16	138
131	92	661	0.16	106
Mean ± S.E.	......	.	0.21 ± 0.05	193 ± 71
		Sept.–Oct.—Dams vitamin D supplemented		
122	82	642	0.21	135
126	100	903	0.21	190
128	90	663	0.05	33
Mean ± S.E.	. ....		0.16 ± 0.05	119 ± 46

[*] Dam fed 200,000 I.U. daily of vitamin D 8 weeks prepartum.

spring. The only statistically significant difference between seasons was the vitamin D content per pound of colostrum.

DISCUSSION

These data, although limited in number and in general exhibiting a high degree of variability, do indicate certain trends.

The prepartum blood plasma levels of vitamin D are in agreement with the work of Light *et al.* (8), Wallis (21), and Hibbs *et al.* (7) in that the level of plasma vitamin D is related to the dietary intake of vitamin D. Seasonal differences in blood plasma vitamin D have been noted earlier by Wallis (21). Wallis (20) has questioned the efficacy of sunlight as being entirely responsible for the higher levels of plasma vitamin D found during the summer months.

The levels of vitamin D in the blood plasma and liver of the newborn calf indicate little storage in these tissues and are suggestive of limited placental transfer at the levels fed. In this experiment, no apparent in-

TABLE 4

Effect of prepartum vitamin D supplementation on colostrum vitamin D

No. of cow	Colostrum		Vitamin D content of colostrum		
	Total 3-day yield	Fat	I.U./g. of fat	I.U./lb. of colostrum	Total I.U. in 3-day yield
	(lb.)	*(%)*			
			April–May—Controls		
101	170	6.8	0.65	20	3409
107	110	3.3	1.06	16	1746
109	165	3.8	1.13	19	3214
111	96	5.2	0.72	17	1630
Mean ± S.E.	180 ± 19	4.8 ± 0.8	0.89 ± 0.12	18 ± 1	2500 ± 471
			April–May—Vitamin D supplemented		
104*	148	4.2	4.00	76	11278
106	165	5.5	3.49	87	14367
108	102	4.0	1.96	36	3627
112	106	5.6	1.66	42	4470
Mean ± S.E.	130 ± 16	4.8 ± 0.4	2.78 ± 0.57	60 ± 12	8436 ± 2616
			Sept.–Oct.—Controls		
127	88	4.8	0.70	15	1341
129	57	2.5	0.83	9	536
131	66	4.4	2.53	50	3332
133	124	4.5	3.18	65	8049
Mean ± S.E.	84 ± 15	4.1 ± 0.5	1.81 ± 0.62	35 ± 13	3314 ± 1684
			Sept.–Oct.—Vitamin D supplemented		
122	48	3.9	5.12	91	4347
126	113	5.5	6.52	163	18381
128	89	6.1	8.12	225	19997
132	107	4.4	2.01	40	4293
Mean ± S.E.	89 ± 15	5.0 ± 0.5	5.44 ± 1.30	130 ± 40	11755 ± 4305

* This cow was fed 200,000 I.U. daily of vitamin D 8 weeks prepartum.

crease was obtained. Since Toverud and Ender (18) have reported that the level of vitamin D contained in livers of stillborn infants is related to the prepartal diet of the mother, it is possible that the level of prepartal supplementation of vitamin D used in this experiment was not sufficiently high to demonstrate an increase. Guerrant *et al.* (4) have suggested that the differences in the vitamin D content of blood plasma and liver at 6 months of age of calves born in September as compared to those levels found in calves born in November were due to prenatal influence. The data reported herein would seem to substantiate this view.

The colostral storage of vitamin D was found to be related to dietary intake of vitamin D. Polskin *et al.* (13) concluded that prepartal feeding of fish liver oil was not an effective way to increase the vitamin D content of mother's milk. They did observe a variable increase in the vitamin D content of milk samples drawn the first 7 to 9 days postpartum from mothers fed prepartum varying amounts of fish liver oil. The level of vitamin D in the milk of the cow has been found to be related to the dietary intake of vitamin D and, in addition, seasonal trends in the vitamin D content of milk have been reported and correlated with the amount of sunshine (22).

The value of prepartal vitamin D supplementation to the cow and the newborn calf was not determined. Hibbs *et al.* (6) have not been successful in preventing milk fever by prepartal supplementation of vitamin D. In other species there have been reports (9, 11, 12, 17) of possible beneficial results to the mother and her newborn.

SUMMARY

The effects of supplementing the dry cow ration with 100,000 I.U. of vitamin D in the form of irradiated yeast once daily for 8 weeks prepartum at two different seasons of the year have been studied. The following results were obtained:

1. An increase in the vitamin D content of the blood plasma was observed in those cows receiving the vitamin D supplement as compared to those levels obtained in the non-supplemented cows.
2. No consisteint differences were found in the vitamin D content of blood plasma and liver of newborn calves from supplemented dams as compared to newborn calves from control dams.
3. The colostral storage of vitamin D was materially increased by prepartal supplementation of vitamin D.
4. There was a seasonal trend in vitamin D content of maternal blood plasma, blood plasma and liver of the newborn calf, and colostrum. The values obtained during the latter part of the pasture season were materially higher than those values obtained at the latter part of the spring feeding period.

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THE EFFECT OF A MASSIVE DOSE AT BIRTH OF VITAMINS A AND D UPON BLOOD PLASMA LEVELS AND LIVER STORAGE IN HOLSTEIN CALVES[1]

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Inasmuch as the newborn calf usually is deficient in vitamins A and D, and colostrum supplies variable amounts of these essentials, it has been suggested that a massive dose of these vitamins at birth might be beneficial to the young calf. Hibbs and Krauss (6) have shown that feeding large amounts of vitamin A on the third and tenth day of age maintained higher-than-normal blood plasma levels and liver storage of vitamin A for a period of 30 days.

The study reported herein was undertaken to determine the effect of a single massive dose of vitamins A and D at birth on the level of carotene, vitamin A, and vitamin D in the blood plasma from birth to 42 days of age, and on the storage of carotene, vitamin A, and vitamin D in the liver at 84 hours of age.

EXPERIMENT I

Animals. Twelve purebred Holstein calves born in the Cornell University herd during March, April, and May, 1946, were used for the studies on blood plasma. They were placed in individual pens at birth. The dams of these calves received similar prepartum treatment. Two vitamin pellets,[2] each containing 125,000 I.U. of vitamin A, 25,000 I.U. of vitamin D, and 250 mg. of niacin chloride, were given by balling gun to alternate calves at the time of the first feeding of colostrum. The feeding schedule was as follows:

Age (days)	*Feed*
1–3	6 lb. of colostrum
4–7	6 lb. of herd milk
8–14	7 lb. of herd milk
15–35	8 lb. of herd milk and free access to calf starter and hay
36–42	6 lb. of herd milk and free access to calf starter and hay

The first feeding was 4 hours after birth and twice daily thereafter. Nipple pails were used for the first 28 days. The colostrum, which was

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[1] This paper is part of a thesis presented by H. D. Eaton to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Manufactured by Standard Brands, Inc., New York, N. Y.

collected from cows fed winter rations, was pooled and held at 0° F. It contained 254 µg. of carotene, 1,143 µg. of vitamin A, and 23 I.U. of vitamin D per lb. The Holstein herd milk was produced under winter feeding conditions. An aliquot of daily samples of this herd milk collected during the course of the experiment contained 48 µg. of carotene, 95 µg. of vitamin A, and 10 I.U. of vitamin D per lb. The hay was predominantly second-cutting U. S. no. 1 timothy and contained 11,105 µg. of carotene and 980 I.U. of vitamin D per lb. The calf starter contained 862 µg. of carotene and 1,347 I.U. of vitamin D per lb. The calves had free access to water at all times.

The possibility of infectious scours was eliminated by giving each calf 4 g. of sulfathalidine twice daily by balling gun for the first 3 days after birth, and once daily for the next 7 days. Later cases of scours were treated by reducing the allowance of milk and feeding a mixture of kaolin, pectin, and sulfathiazole. Incidence and severity of scours were recorded. Calves were weighed at birth and once weekly thereafter.

Samples. Blood samples were obtained at the end of the colostral feeding period (3 days) and at weekly intervals thereafter. These samples were drawn between 7 and 8 a.m. from the jugular vein, and saturated aqueous sodium citrate was used as an anticoagulant. The samples were cooled to approximately 40° F., and all but 2 to 5 ml. (for total hemoglobin determination) were centrifuged at 2,500 r.p.m. for 20 minutes. Vitamin A and carotene and total blood hemoglobin were determined the same morning the samples were drawn. The remainder of the plasma was held at 0° F. until assayed for vitamin D.

Analyses. Blood plasma vitamin A and carotene were determined by the method of Kimble (8), and total blood hemoglobin by the method of Evelyn and Malloy (4). An Evelyn photoelectric macrocolorimeter was used; it previously had been standardized against crystalline carotene in petroleum ether and against crystalline vitamin A in chloroform.

Vitamin D determinations[3] were made according to the rat assay method essentially as set forth in the U. S. Pharmacopoeia XII (17). The approximate level of each type of sample to be fed was determined by preliminary assays. Photographs[4] were made of the stained bones as a matter of record. The vitamin D content was calculated by establishing an equation for a curve of response essentially as outlined by Coward (2).

The data were treated statistically by analysis of variance to determine significance as described by Snedecor (13). Due to the limited number of observations, only those differences significant at the 1 per cent level are reported as statistically significant in the results.

[3] These assays were made by the Laboratory of Industrial Hygiene, Inc., 76 Madison Ave., New York, N. Y., under the direction of Miss Grace McGuire.

[4] Complete records are on file in the Department of Animal Husbandry, Cornell University.

Results. The blood plasma level of carotene, vitamin A, and vitamin D, and the total hemoglobin of whole blood are presented in table 1.

The carotene content was significantly lower throughout the entire experimental period for calves fed the supplementary vitamins. For any one age this difference was not statistically significant. Both groups of calves showed a significant elevation of blood plasma carotene with an increase in age. There was significant variation among calves within a group during the experimental period.

TABLE 1

*The effect of a massive dose at birth of vitamins A and D upon plasma carotene, vitamin A, vitamin D, and total hemoglobin from 3 to 42 days of age**

	Age (days)					
	3	14	21	28	35	42
Carotene—µg./100 ml. plasma						
Controls	6.7 ± 1.5	10.5 ± 2.4	13.7 ± 2.6	14.8 ± 2.3	21.2 ± 2.1	25.8 ± 3.4
Vitamin-supplemented	7.7 ± 2.0	8.5 ± 2.2	12.8 ± 1.9	14.3 ± 1.5	13.0 ± 2.2	17.3 ± 3.4
Vitamin A—µg./100 ml. plasma						
Controls	15.3 ± 2.6	11.5 ± 1.4	12.0 ± 1.7	8.4 ± 1.7	10.4 ± 1.4	10.9 ± 1.4
Vitamin-supplemented .	22.7 ± 3.8	17.1 ± 2.3	12.3 ± 1.2	13.4 ± 2.0	14.3 ± 1.7	14.4 ± 1.8
Vitamin D—I.U./100 ml. plasma						
Controls	67 ± 13		40 ± 11			55 ± 7
Vitamin-supplemented	151 ± 38		148 ± 18			90 ± 12
Total hemoglobin—g./100 ml. whole blood						
Controls	10.46 ± 0.37	10.09 ± 0.38	9.43 ± 0.50	8.24 ± 0.38	8.36 ± 0.40	8.67 ± 0.28
Vitamin-supplemented	10.87 ± 0.45	10.51 ± 0.38	9.50 ± 0.42	8.34 ± 0.39	8.55 ± 0.19	8.82 ± 0.34

* Each value represents the mean ± standard error for 6 calves.

The level of vitamin A in the blood plasma of the vitamin supplemented calves was significantly higher than that of the controls throughout the entire experimental period. At any one age this difference was not statistically significant. Calves within a group varied significantly with age and among individuals.

Blood plasma vitamin D values were significantly higher during the entire experimental period for the vitamin-supplemented calves. For any particular time, the only statistically significant difference was at 21 days of age. Calves within a group did not exhibit statistically significant differences with age or among individuals.

Total hemoglobin was determined to establish some normal values for calves raised on a limited whole milk-dry calf starter method. No significant difference in the total hemoglobin level between groups was observed. Calves decreased significantly in the total hemoglobin level with an increase in age. Significant variation was found among individual calves.

No significant differences were found in the consumption of starter or hay, liveweight increases, and incidence or severity of scours.

EXPERIMENT II

Animals. Six purebred Holstein calves dropped in the Cornell University herd during June, July, and August, 1946, were used in the experiment on storage in the liver. They were placed in individual pens at birth. The dams of these calves received similar prepartum treatment. Two vitamin pellets identical to those previously described were given by balling gun to alternate calves at the time of the first feeding of colostrum. The feeding schedule was as follows:

Age (hours)	*Feed**
6	3 lb. colostrum
18	3 lb. colostrum
30	3 lb. colostrum
42	3 lb. colostrum
54	3 lb. reconstituted skim milk
66	3 lb. reconstituted skim milk
84	Calf slaughtered

* Calves nos. 2 and 11 were fed colostrum and reconstituted skim milk in amounts representing 8 per cent of their liveweight at birth.

Nipple pails were used throughout the experiment. The colostrum, which was collected from Holstein cows which had been on pasture prior to calving, was pooled and held at 0° F. This colostrum contained 528 μg. of carotene, 504 μg. of vitamin A, and 24 I.U. of vitamin D per lb. Carotene and vitamin A were not detected in the dried skim milk.

Samples. Blood samples were drawn before each feeding and at the time of slaughter. Treatment of these samples was identical to that described in experiment I.

The livers were cooled to 38° F., cut into small pieces, and placed on an enamel tray. Aliquots were taken for carotene and vitamin A analyses; the tray then was placed in an oven which had a constant circulation of air provided by an electric fan, and held at 60° C. for 24 hours. The livers then were removed from the oven, allowed to reach a constant weight at room temperature, ground in a Wiley mill, and held at 38° F. until assayed for vitamin D.

Analyses. The analytical methods and statistical treatments used were identical to those described in experiment I. The carotene and vitamin A contents of the livers were determined by the method of Davies (3) within 48 hours after slaughter.

TABLE 2

*The effect of a massive dose at birth of vitamins A and D upon plasma carotene and plasma vitamin A from 6 to 84 hours of age**

Age	Carotene µg./100 ml. plasma		Vitamin A µg./100 ml. plasma	
	Controls	Vitamin-supplemented	Controls	Vitamin-supplemented
(*hr.*)				
6	3.0 ± 1.0	2.3 ± 1.5	5.3 ± 3.0	5.7 ± 3.1
18	4.7 ± 0.9	4.7 ± 1.8	8.4 ± 4.9	24.6 ± 15.3
30	9.3 ± 1.3	9.7 ± 3.5	10.5 ± 5.4	29.7 ± 18.0
42	13.0 ± 2.1	13.7 ± 3.0	12.6 ± 6.6	17.6 ± 9.2
54	17.0 ± 1.0	18.7 ± 3.5	15.1 ± 8.4	30.7 ± 20.7
66	13.7 ± 0.9	19.0 ± 2.5	11.7 ± 6.3	89.8 ± 48.0
84	14.7 ± 1.8	16.3 ± 2.6	9.6 ± 5.3	28.9 ± 15.4

* Each value represents the mean ± standard error for 3 calves.

Results. The level of carotene and vitamin A in the blood plasma is presented in table 2. Plasma carotene was not significantly different between groups. There was a tendency for the level of carotene in the plasma to drop after the feeding of reconstituted skim milk. Calves within a group varied significantly in plasma carotene levels. The level of vitamin A in the blood plasma was significantly higher in the vitamin-supplemented calves for the entire experimental period. A marked elevation in plasma vitamin A resulted in the vitamin-supplemented calves after a feeding of reconstituted skim milk. Calves within a group showed significant variation with age and among individuals.

The mean blood plasma level of vitamin D at 84 hours of age was 42 ± 13 I.U. for the controls and 154 ± 27 I.U. for the vitamin-supplemented calves. This difference, although marked, was not statistically significant.

The carotene, vitamin A, and vitamin D contents of the livers are presented in table 3. Liver carotene was approximately the same for both

TABLE 3

*The effect of a massive dose at birth of vitamins A and D upon the liver carotene, liver vitamin A, and liver vitamin D at 84 hours of age**

Carotene†		Vitamin A†			Vitamin D†	
µg./g. liver	Total µg. in liver	µg./g. liver	Total µg. in liver	Total I.U. in liver‡	I.U./g. liver	Total I.U. in liver
			Controls			
0.53 ± 0.07	421 ± 78	4.26 ± 0.86	3267 ± 623	13770 ± 2398	0.14 ± 0.04	110 ± 26
			Vitamin supplemented			
0.63 ± 0.12	442 ± 74	62.01 ± 5.96	43579 ± 2147	175054 ± 8709	2.24 ± 0.21	1606 ± 235

* Each value represents the mean ± standard error for 3 calves.

† Expressed on a wet basis.

‡ Assuming 0.6 µg. carotene = 1 I.U. of vitamin A and 0.25 µg. vitamin A = 1 I.U. of vitamin A.

groups of calves. The levels of vitamin A and vitamin D were ten times higher in the vitamin D-supplemented calves than in the control calves, and these differences were statistically significant.

DISCUSSION

These data indicate that the blood plasma level of vitamins A and D may be maintained at a level higher than normal by the administration of a massive dose of these vitamins at birth to the Holstein calf. The apparent reason for this is the relatively high liver storage obtained after the administration of these vitamins.

The general trends of the levels of carotene and vitamin A in the blood plasma of the control calves are in agreement with those reported by Moore and Berry (11, 12) for Holstein calves.

The depression of the carotene level in the blood plasma from 3 to 42 days of age in those calves fed the vitamin supplement has been observed previously in lactating cows fed relatively high doses of vitamin A. This recently has been discussed by Wise *et al.* (18). The mechanism involved is yet unknown. Apparently considerable time is necessary for the factor or factors which cause this depression in plasma carotene to act, for no such depression was noted until the second week after administration of the large dose of vitamin A.

The maintenance of higher-than-normal blood plasma levels of vitamin A in the vitamin-supplemented calves is in agreement with the work of Sutton and Kaeser (16) and Hibbs and Krauss (6). A massive dose of vitamins at birth is as effective as daily doses or two massive doses at 3 and 10 days of age in maintaining high blood plasma levels of vitamin A.

After a feeding of reconstituted skim milk, there was a marked elevation in the blood plasma level of vitamin A in those calves receiving the vitamin supplement. Apparently at high levels of liver storage of vitamin A there is a mobilization of the vitamin A from the liver and other depots into the blood stream.

By the administration of a single massive dose of vitamin A at birth to the calf it is possible to obtain greater liver storage of total vitamin A than that obtained by prepartal feeding, as reported by Spielman *et al.* (15). Although the administration of vitamins at birth is more economical, the possible benefit to the calf in its intrauterine development through prepartum supplementation of its dam with vitamin A should not be overlooked. Studies by Hibbs and Krauss (6) and Spielman *et al.* (14) have shown that massive doses of vitamins have not resulted in improved performance.

It should be noted that the vitamin pills used contained niacin chloride. The influence of the sparing action of niacin on vitamin A (10) may be disregarded, since recently Johnson *et al.* (7) have found niacin to be unessential in the diet of the calf. Moreover, Hibbs and Krauss (6) have reported no effect on blood plasma levels or liver storage of vitamin A with the daily addition to the diet of 50 mg. of niacin.

The small recovery of vitamin D in the livers of the vitamin-supplemented calves is in agreement with the work of Guerrant *et al.* (5). These workers found relatively small differences in liver storage of vitamin D with wide difference in dietary intake of vitamin D. A possible loss of vitamin D in the oven-drying method has been investigated by Loosli *et al.* (9). Preliminary evidence indicates no appreciable loss between oven-dried and freeze-dried liver.

The decrease in total hemoglobin with an increase in age in young calves has been observed previously by Anderson *et al.* (1) and by others.

SUMMARY

The effect of a massive dose of vitamins A and D fed to Holstein calves at birth has been studied. The following results were obtained:

1. A significant depression in the levels of carotene in the blood plasma occurred in those calves which received the vitamin supplement as compared to those in the control group. This depression was apparent 2 weeks after the administration of the massive dose of vitamins and continued until the end of the experimental period at 6 weeks of age. The levels of both vitamin A and vitamin D in the blood plasma of the vitamin-supplemented calves were significantly higher than those levels for the control calves.
2. The storage of vitamins A and D in the livers of the vitamin-supplemented calves was ten times higher than that found in the control calves. No appreciable differences in liver carotene storage were observed.

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A STUDY OF THE CHANGES OCCURRING IN THE FAT CONSTANTS OF MILK FAT OF CHEDDAR CHEESE DURING THE RIPENING PROCESS

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The determination of the chemical changes occurring in the various constituents of Cheddar cheese and their effect on flavor development during ripening have been the object of numerous investigations. The effects on the flavor of the changes in the various nitrogenous fractions, the rate and amount of acid development, production of volatile acids, and the microflora of the cheese have received considerable attention. Less attention has been directed toward determining changes occurring in the cheese fat than in the protein fraction.

A survey of the literature did not reveal any studies pertaining to the changes occurring in the fat constants of cheese fat during the ripening process comparable with studies made on butterfat. Therefore, the purpose of the investigation reported herein was to determine the relationship between changes in the fat constants of cheese fat and flavor development of the cheese. The problem was divided into three parts to determine the effect of the following factors on changes in the fat constant values and flavor development: (a) pasteurization of the milk, (b) homogenization of the milk, and (c) the addition of organic acids to the milk and cheese curd.

REVIEW OF THE LITERATURE

The literature on cheese ripening is so extensive that only those papers pertaining to chemical changes in cheese fat will be reported. As early as 1891 Van Slyke (11) recognized that the fat of cheese changed in flavor during ripening. It was his opinion that the change in the fat was primarily hydrolytic, releasing free fatty acids. These later combined with ammonia, liberated from protein hydrolysis, to form ammonium salts of the acids. Suzuki *et al.* (10) in 1910 showed that the non-nitrogenous products

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[1] This paper represents a portion of the thesis presented by the senior author in partial fulfillment of the requirements for the M.S. degree at the University of Illinois. The research was completed before the authors entered the armed forces in 1942.

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formed during the ripening of Cheddar cheese contributed appreciably to its flavor. These investigators were the first to partition, by the Duclaux technique, the volatile acids obtained from steam distillation of Cheddar cheese. Furthermore, their results showed that: (a) Acetic and propionic acids reached a maximum at 3 months and then decreased, whereas butyric and caproic acids continually increased throughout the experimental period of 10 months. (b) The principal source of acetic and propionic acids probably was lactates, with protein and glycerine contributing only small amounts. (c) The principal sources of butyric and caproic acids were the fat and the protein. Suzuki *et al.* (10) produced a distillate having the aroma of aged cheese and containing alcohols and esters. The alcohol was primarily ethyl with esters of acetic acid and ethyl alcohol. Skim milk cheese distillate contained the esters of butyric and caproic acids and ethyl alcohol.

Lane and Hammer (6) showed that as cheese ripened the acidity of the fat increased. Furthermore, the acid values of the fat from raw milk cheese normally were higher than those of fat from cheese made from pasteurized milk. Lane and Hammer (7) later published results demonstrating that cheese made from milk standardized with raw homogenized cream and raw or pasteurized skim milk commonly developed a rancid flavor which gradually disappeared during ripening. The resulting flavor of the ripened cheese was typical. The fat acidity of this cheese was higher and its flavor more pronounced than that of cheese made from pasteurized, homogenized cream and pasteurized skim milk. However, when the rancidity was induced by pancreatin, this flavor did not disappear during ripening and the resulting product was undesirable. The addition of desiccated mammary tissue or extracts of them to pasteurized milk commonly had a desirable effect on the flavor of the cheese. Although slight rancidity occurred during the early period of ripening, it finally disappeared, producing a desirable Cheddar cheese flavor. The fat acidity was higher than that of the control, suggesting some relationship between fat acidity and development of desirable flavor. However, the authors state that fat acidity is not always a true index of a typical and desirable flavor in cheese for ". . . perhaps changes other than simple hydrolysis are necessary in the development of the typical flavor."

Babel and Hammer (2) in 1945 continued the study of fat hydrolysis in cheese and showed that, without the addition of lipase, the acidity of the fat of pasteurized milk cheese increased during the ripening period. With the addition of rennet paste, the fat acidity increased and a rancid flavor developed which frequently disappeared during ripening, leaving a desirable cheese. However, the rancidity induced by the lipase of mulberry juice when added to pasteurized milk in sufficient quantities to promote hydrolysis of the fat produced unclean flavors in the cheese.

In an attempt to standardize and determine the validity of the results of certain chemical procedures which have been used to secure the volatile acids of cheese, Hiscox and Harrison (4) made a critical study of various techniques. Their results showed that direct steam distillation of the cheese did not give a true figure for the volatile acids present in cheese, since cheese fat retarded distillation and cheese protein produced a definite retention of some of the acids. A more accurate figure could be obtained by distilling to twice the original volume than by distilling to five times the original volume. Only butyric acid could be recovered completely, regardless of the amount of distillate.

Hiscox *et al.* (5) then recommended a procedure based on water extraction of the fatty acids from cheese and ether extraction of the fat and fat-soluble fatty acids, the latter being removed by washing the ether with dilute alkali. The acids in the extractions were freed by acidifying and steam distilling.

Marquardt and Dahlberg (8), following the work of Hiscox and Harrison (4), standardized the size of cheese sample distilled and the volume of water used for distillation. Since the completion of the experimental work reported in the present paper, Smiley *et al.* (9) have simplified the procedure of Hiscox *et al.* (5). Their method employs two distillations of acid cheese solution—one associated with the fat phase and the other with the residual cheese material. The total volatile acidity is the sum of the titrations for the two distillations plus the alcohol used to rinse the condenser. Results are expressed as ml. of N/10 acid per 100 g. of cheese. The authors showed good agreement in comparing results obtained by the simplified extraction and the method of Hiscox *et al.* (5).

EXPERIMENTAL PROCEDURES

Cheese Manufacturing Methods. Fourteen samples of cheese were made and analyzed. The milk used for all the cheese was of high quality, being obtained from the University dairy herd. The different lots of milk varied in weight from 250 to 3,000 lb. The milk was clarified, tested for fat (3.8 per cent average), pasteurized in a spray vat at 143° F. for 30 minutes, and cooled to the setting temperature for cheesemaking. The manufacturing methods were such as to make a low-acid type of cheese containing 35–36 per cent moisture. However, when organic acids were added to the milk or to cheese curd, undesirable acid defects developed. The cheese was ripened at 50° F. Samples were taken for analysis at 30-day intervals, at which time the cheese also was judged organoleptically.

Chemical Methods of Analysis. The samples of fat used for chemical analysis were obtained by petroleum ether extraction of the cheese in a Soxhlet extraction apparatus. Sufficient cheese was extracted over a 4- to 6-hour period to furnish enough fat to make the various determinations. The following determinations were made on the cheese fat:

a. The acid number was determined by the method discussed by Breazeale and Bird (3) and listed as modification A of the alcoholic potassium hydroxide titration method. The acid number is expressed as the number of ml. of N/10 alkali per 10 g. of fat.

b. Reichert-Meissl number, Polenske number, saponification number, iodine number (Part III), and percentages of soluble acids (as butyric), and insoluble acids were determined by the methods of the Association of Official Agricultural Chemists (1).

c. Total volatile acidity of cheese was determined by the method of Hiscox and Harrison (4) as modified by Marquardt and Dahlberg (8). It was known that lower values would be obtained than if the refined method of Hiscox *et al.* (5) had been used. The value is expressed as the number ml. of N/10 NaOH per 200 g. of cheese.

RESULTS AND DISCUSSION

To simplify the presentation of data and results, the problem will be discussed in three parts.

Part I. The Effect of Pasteurization of the Milk upon the Fat Constant Values of Cheese Fat

To determine the effect of pasteurization of the milk on the rate of change in the values of the fat constants, four lots of cheese were made. Lots 1 and 2 represent raw milk and pasteurized milk cheese, respectively, from the same source of milk. Lots 3 and 4 also are raw milk and pasteurized milk cheese, respectively, from the same source of milk.

The analytical data of the fat for these lots of cheese are presented in table 1. The scores for organoleptic analysis of the samples are given in table 2.

From observation of the data in table 1, on the acid number values, it is apparent that there is a consistent increase for all cheese samples throughout the aging period. The percentage increase in acid numbers for the four lots was as follows: 1, 105; 2, 100; 3, 120; and 4, 87. Other workers also have found this change to occur in cheese fat. In agreement with the results of Lane and Hammer (6), the lots of raw milk cheese had higher acid numbers than did the pasteurized milk lots. Lot 1 had an acid number 55 per cent greater than lot 2. However, the acid number of lot 3 was only 20 per cent greater than for lot 4.

The Reichert-Meissl (RM) value represents a measure of the amount of volatile and water-soluble fatty acids in the fat. These consist mainly of butyric, caproic, and caprylic acids. If the fat showed appreciable hydrolysis during the ripening period, the normal trend would be for the RM number to increase. Suzuki *et al.* (10) showed that butyric and caproic acids increased throughout the ripening period in their raw milk cheese.

TABLE 1

Fat constants of cheese made from raw and pasteurized milk

Time (days)	Acid nos.				Reichert-Meissl nos.[a]				Polenske nos.			
	Lot 1[a]	Lot 2[b]	Lot 3[a]	Lot 4[b]	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4
1	1.47	0.97	1.12	1.13	28.80	28.70	29.41	29.92	2.67	2.83	2.70	2.24
30	1.78	1.09	1.40	1.16	...	...	...	...	...	...	...	...
60	1.43	1.19	1.52	1.28	29.35	28.65	29.65	28.58	3.47	3.42	2.46	2.00
90	1.24	1.30	1.81	1.48	...	...	...	...	...	...	...	...
120	1.55	1.33	1.89	1.56	28.80	27.95	28.95	28.50	2.45	2.40	2.47	2.00
150	1.80	1.39	2.12	1.79	...	...	...	...	...	...	...	...
180	2.10	1.43	2.16	1.85	29.97	28.80	27.50	28.25	2.35	2.76	1.82	1.64
210	2.26	1.42	2.32	1.95	...	...	...	...	...	...	...	...
240	2.86	1.49	2.42	2.07	29.93	28.75	27.40	27.15	2.20	2.25	1.77	1.55
270	3.02	1.94	2.46	2.12	...	...	...	...	...	...	...	...

Time (days)	Percentage of soluble acids				Percentage of insoluble acids				Saponification nos.			
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4
1	5.03	4.80	3.82	3.85	81.30	82.00	82.20	82.50	229.90	230.00	228.00	228.00
30	...	...	...	...	...	...	...	...	...	...	...	...
60	5.89	5.50	5.78	4.70	79.80	85.00	80.70	83.00	228.10	228.30	226.70	225.80
90	...	...	...	...	...	...	...	...	225.70	224.00	223.80	225.00
120	4.15	4.15	4.30	4.05	79.00	82.05	82.20	84.30	...	...	...	...
150	...	...	...	...	...	...	...	...	225.00	226.60	225.90	224.50
180	4.20	4.25	4.54	4.15	77.70	83.10	83.60	82.80	224.15	226.90	228.00	221.40
210	...	...	...	...	...	...	...	...	...	...	...	...
240	4.10	4.10	4.40	4.25	76.60	78.80	80.70	81.00	223.30	223.70	229.00	221.40
270	...	...	...	...	...	...	...	...	220.00	221.10	229.00	221.00

[a] Raw milk cheese.

[b] Pasteurized milk cheese.

Lot 1 also demonstrated this by the increase in RM number. Lot 2 showed little change in Reichert-Meissl value, indicating only slight hydrolysis. The flavor score at 270 days was 0.5 point in favor of lot 1, the raw milk cheese which showed a slight amount of fat hydrolysis. The trend of the RM values for lots 3 and 4 was downward, instead of upward as might be expected.

The Polenske number is a measure of the volatile fatty acids, capric, lauric, and myristic, which are insoluble in water but soluble in neutral 95 per cent alcohol. The four lots of experimental cheese all showed a significantly lower Polenske value at the conclusion of the experiment, indicating a decrease in the insoluble volatile fatty acids. However, lots 1 and 2 demonstrated an increase in Polenske number at 60 days, which was followed by a decrease in value during the remainder of the period. This perhaps showed that the fresh cheese fat was first hydrolyzed, followed by a hydrolysis of the longer chain fatty acids.

TABLE 2

Flavor and body scores and criticisms of cheese made from raw and pasteurized milk

Time (days)	Lot 1[a]		Lot 2[b]		Lot 3[a]		Lot 4[b]	
	Score	Criticisms	Score	Criticisms	Score	Criticisms	Score	Criticisms
Flavor scores and criticisms								
30	40.0	....	40.0		40.0		40.0	. .
180	40.0	. ..	40.0	. .	38.5	Sl. bitter	40.0	.
210	39.5	.	39.5	.	38.5	Sl. bitter	40.0	
270	39.5		39.0	..	39.5		38.5	Sl. acidy
Body scores and criticisms								
30	29.0	. .	28.5	Sl. curdy	29.0	Curdy	29.0	.
180	29.0		29.0		28.5	Curdy	28.5	Sl. mealy
210	29.0	.	28.5	Sl. mealy	28.5	Curdy	28.5	Sl. mealy
270	29.0	..	28.5	Sl. mealy	29.0		28.5	Sl. mealy

[a] Raw milk cheese.
[b] Pasteurized milk cheese.

A fat which becomes hydrolyzed should show a progressive increase in soluble acids as hydrolysis proceeds, along with a decrease of insoluble acids or Hehner value. The cheese fat from each of the four lots demonstrated an increase in soluble acids at 60 days. However, at 240 days the final values for soluble acids in lots 1 and 2 were less than at the beginning of ripening. In lots 3 and 4, even though there was a consistent decrease in quantity of soluble acids, the change did not occur as rapidly as in lots 1 and 2; consequently, the final values were somewhat greater than they were at the start of the period. At 240 days, the percentages of insoluble acids in all four lots were less than at the beginning of the period. This gives further evidence that the longer chain fatty acids were hydrolyzed during ripening of cheese.

Since the saponification number represents the number of milligrams of potassium hydroxide required to saponify 1 g. of fat, then glycerides containing low molecular weight fatty acids will have a greater saponification number than glycerides containing fatty acids of high molecular weight. Except for lot 3, the cheese fat showed a decrease in saponification numbers during the ripening period, indicating a decrease in number of low molecular weight fatty acids in the cheese fat.

An examination of the flavor scores of the cheese in table 2 shows that all cheese samples were of high quality at the beginning of the experiment. At the end of 270 days, however, the flavor of the cheese in lots 1 and 3 was slightly more pleasing. The scores for lots 1 and 3 were 0.5 and 1 point higher than for lots 2 and 4. The fat constant values for lot 1 clearly indicated a slow hydrolysis of the butterfat. This trend was not so well defined in the other samples.

Part II. The Effect of Low Pressure Homogenization of the Milk on the Change in Values of the Fat Constants

The homogenization of raw cream used to standardize pasteurized skim milk increases the acid number of the cheese fat (7). Homogenization of pasteurized milk at high pressures produces a soft curd when the milk is coagulated by rennin or pepsin. To ascertain the effect of low pressure homogenization on the fat constant values and on the body of the cheese when made from pasteurized whole milk, three lots of cheese were made.

A vat of pasteurized milk was divided into three batches and treated as follows: (a) Batch 1 was homogenized at 300 lb. pressure at 143° F. through a 200-gallon-per-hr. Multi-Flo Creamery Package homogenizer, cooled, and then made into the cheese constituting lot 5. (b) Batch 2 was treated in a similar manner except that the pressure was increased to 800 lb. Cheese lot 6 was made from this milk. (c) Batch 3 was the control without homogenization and was made into the cheese of lot 7.

All the analytical data of the fat analyses are given in table 3. The results of the organoleptic judging of body and texture are given in table 4. Observation of the data presented in table 3 shows that the acid number of the fat in all lots increased throughout the ripening period. The increase was as follows: Lot 5, 50 per cent; lot 6, 44 per cent; lot 7, 63 per cent. This increase is considerably less than was noted for the samples in Part I.

The RM numbers decreased for the fat in lots 5 and 6, made from pasteurized, homogenized milk, although the RM value slightly increased for the fat in lot 7. An increase in RM number should occur if volatile, water-soluble fatty acids were liberated.

The Polenske numbers for the fat from the different lots of cheese de-

TABLE 3

Fat constants of cheese made from homogenized and unhomogenized milk

Time (days)	Acid nos.			Reichert Meissl nos.			Polenske nos.		
	Lot 5[a]	Lot 6[b]	Lot 7[c]	Lot 5	Lot 6	Lot 7	Lot 5	Lot 6	Lot 7
1	1.76	1.66	1.55	29.50	29.30	27.63	2.02	2.26	2.00
30	1.85	1.68	1.73						
60	1.89	1.72	1.83	28.50	29.25	28.01	1.85	2.50	1.97
90	1.96	1.82	2.10						
120	2.34	2.05	2.32	27.70	27.30	28.10	1.91	1.91	1.82
270	2.65	2.40	2.54	27.60	27.00	28.10	1.50	1.84	1.64

Time (days)	Percentage of soluble acids			Percentage of insoluble acids			Saponification nos.		
	Lot 5	Lot 6	Lot 7	Lot 5	Lot 6	Lot 7	Lot 5	Lot 6	Lot 7
1	5.32	5.80	5.10	81.05	84.10	82.41	227.21	227.81	221.34
30									
60	4.40	4.73	5.15	80.60	82.30	81.23	228.26	226.29	222.68
90									
120	4.62	4.62	4.23	82.50	85.72	80.16	227.74	227.83	226.53
270	4.40	4.42	4.21	83.00	82.13	79.17	228.38	228.13	226.77

[a] Homogenized at 300 lb. pressure at 143° F. through a 200 gallon per hr. Multi Flo Creamery Package homogenizer, cooled, and made into cheese.

[b] Treated in a manner similar to lot 5, but homogenized at 800 lb. pressure.

[c] Control, not homogenized.

creased during the ripening period, but in lot 6 there occurred an increase in Polenske value at 60 days, followed by a decrease. This condition also took place in lots 1 and 2 in Part I.

TABLE 4

Flavor and body scores and criticisms of cheese made from homogenized and unhomogenized milk

Time (days)	Lot 5[a]		Lot 6[b]		Lot 7[c]	
	Score	Criticisms	Score	Criticisms	Score	Criticisms
	Flavor scores and criticisms					
1	39.0		39.0		39.0	
60	38.0	Sl. cooked	38.0	Sl. cooked	38.5	Sl. bitter
120	38.5	Sl. acidy	40.0		38.0	Bitter[a]
270	39.0		38.0	Sl. acidy Sl. bitter	38.5	Sl. bitter
	Body scores and criticisms					
1	29.0		28.5	Sl. curdy	29.0	
60	28.5	Sl. weak	28.5	Sl. curdy	29.0	
120	29.0	Sl. open	29.5		29.0	
270	28.5	Sl. mealy	28.0	Sl. pasty	28.5	Sl. weak

[a] Homogenized at 300 lb. pressure at 143° F. through a 200-gallon-per-hr. Multi-Flo Creamery Package homogenizer, cooled, and made into cheese.

[b] Treated in a manner similar to lot 5 but homogenized at 800 lb. pressure.

[c] Control, not homogenized.

The trend of the values for both the soluble and insoluble acids was downward. The percentage of insoluble acids would be expected to decrease in proportion to the amount of hydrolysis occurring in the longer chain fatty acids. Moreover, the soluble acids might be expected to increase as a result. The decrease, however, in percentage of soluble acids for lots 5 and 6 is in agreement with the trend of the RM numbers for these samples.

The saponification numbers of the fat from lots 5, 6, and 7 showed an increase during the ripening period. The reverse of this trend occurred with lots 1, 2, 3, and 4.

An examination of the flavor scores for lots 5, 6, and 7 (table 4) indicates that the flavor of lot 5 was superior to the others at the end of 270 days. Lot 6 was the least desirable, for it had a bitter flavor and a pasty body. There was little difference, however, between lots 5 and 7.

Except for the acid numbers and Polenske numbers, the other fat constant values do not present clear evidence of significant fat hydrolysis.

Part III. The Effect of the Addition of Various Organic Acids upon the Fat Constant Values and Volatile Acidity and Flavor of Cheese

To determine the effect of the addition of various organic acids upon the fat constant values, volatile acidity of the cheese, and flavor of the cheese, seven lots were made. Two-hundred fifty pounds of pasteurized milk were used for each lot and the substances added were as follows: Lot 8, control; lot 9, 20 ml. of propionic acid added to the milk at time of setting; lot 10, 15 ml. of glacial acetic acid added to the curd at time of salting; lot 11, 15 ml. of glacial acetic acid added to the milk at time of setting; lot 12, 1 l. of 2 per cent citric acid added to the milk at time of setting; lot 13, 1.5 pints of starter distillate added to the milk at time of setting and 0.5 pint of starter distillate to the curd at salting; lot 14, 10 ml. of propionic acid, 10 g. of citric acid, and 14 ml. of glacial acetic acid dissolved in 1 l. of water added to the milk at setting. The different lots of cheese were not made from the same milk. However, during the period of time the cheese was made, no significant changes occurred in source of milk.

The analytical data for the fat constants of the seven lots are presented in table 5. The judging scores for the various lots are listed in table 6. Observation of the data on acid numbers of the cheese fat, as given in table 5, shows the same trend as those samples in Parts I and II. The percentage increase in acid values for the samples was as follows: Lot 8, 60; lot 9, 67; lot 10, 218; lot 11, 73; lot 12, 71; lot 13, 42; and lot 14, 76. A marked effect was produced in lot 10 when acetic acid was added to the curd at time of salting. The hydrolysis of the fat was activated to a pronounced degree, for by the end of the experiment an increase of over 300 per cent in acid value had occurred. When acetic acid was added to the milk in lot 11, the acid number of the fat was high at the beginning, so the percentage increase

Fat constants of Cheddar cheese containing added organic acids and starter distillate

Time (days)	Acid nos.							Reichert Meissl nos.							Polenske nos.						
	Lot 8[a]	Lot 9[b]	Lot 10[c]	Lot 11[d]	Lot 12[e]	Lot 13[f]	Lot 14[g]	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	1.74	0.94	0.90	1.72	0.83	1.73	0.88	27.55	27.36	29.05	27.70	26.30	27.40	27.93	2.16	1.78	2.13	2.06	1.75	2.09	1.84
45	1.81	1.05	1.22	1.98	1.25	1.83	1.13	27.32	27.20	27.90	27.22	26.22	27.80	28.02	1.96	1.82	2.32	2.19	1.85	1.79	1.91
90	1.98	1.17	1.40	1.90	1.12	1.82	1.26	27.50	26.27	27.35	28.30	26.75	27.95	26.65	1.96	1.70	2.13	1.75	1.79	1.55	1.93
135	2.18	1.19	1.43	2.16	1.13	2.09	1.20	28.68	27.30	27.30	28.65	27.90	29.05	25.82	1.85	1.72	2.10	1.68	1.84	1.78	1.72
225	2.44	1.26	1.58	2.40	1.35	2.18	1.41	29.85	27.70	29.22	28.65	27.70	29.50	27.40	1.74	1.90	1.55	1.54	1.74	2.01	1.92
270	2.79	1.57	2.87	2.98	1.42	2.47	1.55	29.92	28.00	29.35	28.93	27.46	29.62	28.75	1.72	1.76	1.43	1.63	1.64	2.38	1.97

Time (days)	Percentage of soluble acids							Percentage of insoluble acids						
	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	2.10	4.30	2.84	3.37	2.90	3.60	3.40	85.52	84.31	82.56	83.73	84.71	82.47	87.72
45	2.67	4.40	2.98	3.68	2.88	3.97	3.58	84.31	86.62	82.03	85.52	84.02	84.40	86.12
90	3.18	4.56	3.16	3.97	3.16	4.05	3.62	84.18	85.04	82.14	85.43	85.68	84.62	84.92
135	3.05	4.40	4.47	4.90	4.04	4.10	3.78	84.14	84.26	82.19	84.89	82.61	84.72	84.18
225	4.82	4.76	5.19	5.82	4.03	4.82	3.91	84.02	83.19	82.33	83.92	80.98	84.13	85.13
270	5.23	4.68	6.18	6.28	4.15	4.71	4.02	83.65	80.63	82.41	83.64	78.52	83.78	86.16

Time (days)	Saponification nos.							Iodine nos.						
	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	222.73	222.16	221.42	222.18	222.24	222.18	222.72	29.62	32.71	33.42	30.82	33.99	30.04	35.52
45	222.98	221.13	223.16	223.52	220.16	221.15	220.32							
90	221.53	221.10	225.18	225.19	220.98	221.74	220.18							
135	222.64	220.52	228.16	226.89	223.64	222.34	223.13	28.81	32.18	34.10	26.83	33.86	29.55	35.74
225	222.98	223.18	228.03	228.53	225.18	221.19	226.43							
270	223.13	224.19	223.45	228.55	226.17	221.12	228.87	27.74	32.03	31.64	25.74	31.64	28.81	32.05

Time (days)	Volatile acid nos.						
	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 14
1	12.04	8.05	19.42	10.70	7.92	12.34	10.42
45	16.50	10.20	19.88	17.25	10.34	16.50	10.33
90	14.50	11.80	19.61	20.90	12.55	18.12	10.43
135	12.20	11.60	17.02	16.83	11.56	17.64	10.22
225	9.80	8.60	14.81	12.89	9.02	16.88	9.68
270	8.68	6.90	13.24	11.40	9.24	14.96	8.44

[a] Control.

[b] 20 ml. of propionic acid added to the milk at time of setting.

[c] 15 ml. of glacial acetic acid added to the curd at time of salting.

[d] 15 ml. of glacial acetic acid added to the milk at time of setting.

[e] 1 l. of 2% citric acid solution added to the milk before adding rennet.

[f] 1.5 pints of starter distillate added at the time of adding rennet and 0.5 pint of starter distillate added to the curd at time of salting.

[g] 10 ml. of propionic acid, 10 g. of citric acid, and 14 ml. of glacial acetic acid in 1 l. of water added at the time of setting.

during ripening was less marked. Lot 13, containing the starter distillate, showed a high acid number at the beginning of the period. The smallest increase during ripening occurred in this sample. The control (lot 8) also showed a high initial acid number.

In general, the water-soluble, volatile acids of the fat for all samples increased during the ripening period. This condition also is confirmed by the increase in RM values as listed in table 5. The increase in RM values was more consistent for the samples in Part III than for samples in Parts I and II. However, the RM values for lot 10 decreased during a 3-month period and then increased again during the last 135 days of ripening.

The Polenske numbers tended to decrease for the majority of samples during ripening. This trend would indicate a decrease in quantity of insoluble, volatile acids. Since the volatile, soluble acids increased at the same time, apparently the glycerides containing long-chain fatty acids were hydrolyzed.

The percentages of soluble acids in all lots of cheese increased throughout the ripening period, presenting further evidence of fat hydrolysis and liberation of low molecular weight fatty acids. The percentages of insoluble acids showed a tendency to decrease, although it is not as well defined as the trend of the soluble acids. The saponification numbers for the different lots showed a tendency to increase. The data on the changes of the iodine number of the cheese fat are inconclusive.

The total volatile acidity of the cheese reached a maximum value, except for lots 8 and 13, at 90 days. Suzuki *et al.* (10) demonstrated that acetic acid and propionic acid reached a maximum value at 90 days and then decreased. The experimental lots of cheese, 10, 11, 13, had the greatest volatile acidity. Lots 10 and 11 contained acetic acid. The drop in volatile acidity is especially apparent at the time the samples were analyzed at 225 days.

At the end of the experiment, samples 12 and 13, to which had been added citric acid and starter distillate, respectively, had the highest flavor score (table 6). The samples containing the added propionic acid and acetic acid showed abnormal and undesirable bitter flavors from the beginning of the experiment. Lot 10, in particular, quickly developed into an undergrade cheese. Due perhaps to the abnormally high amounts of organic acids added to the milk and cheese, their effects on flavor were so pronounced that desirable effects that might have been produced with smaller quantities quickly were masked by the flavor defects which developed.

SUMMARY AND CONCLUSIONS

A study has been made of the changes occurring in the fat-constant values of cheese fat during the ripening of Cheddar cheese. Fourteen lots were made and analyzed at regular intervals during an aging period of 9 months. The study was divided into three parts to determine the effect of

TABLE 6

Flavor and body scores and criticisms of Cheddar cheese containing added organic acids and starter distillate

Time (days)	Lot 8[a]		Lot 9		Lot 10		Lot 11		Lot 12		Lot 13		Lot 14	
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism
							Flavor scores and criticisms							
1	39.5		37.0	Propionic	37.0	Bitter	39.0		39.0		39.5		37.5	Fruity
45	39.0		37.5	Pro. fruity	36.0	Bitter	39.0		38.5	Sl. cooked	39.0		37.5	Acidy
90	39.0		37.5	Propionic	36.0	Bitter	38.0	Bitter	38.5	Sl. cooked	39.0		37.0	Fruity
135	39.0		37.0	Pro. bitter	36.0	Bitter	38.5	Bitter	38.5	Sl. bitter	38.0	Acidy	37.5	Bitter
225	38.0	Sl. bitter	37.0	Pro. fruity	35.0	Bitter	37.5	Bitter	38.5	Sl. bitter	38.5	Sl. acidy	37.5	Bitter
270	37.5	Bitter	37.5	Pro. fruity	35.0	Bitter	37.0	Bitter	38.5	Sl. bitter	38.5	Sl. acidy	37.0	Bitter
							Body scores and criticisms							
1	29.0		28.5	Sl. curdy	27.0	Soft	29.0		28.5	Sl. curdy	29.0		28.5	Weak
45	29.0		28.0	Weak, curdy	27.0	Soft	29.0		28.0	Curdy	29.0		27.0	Weak
90	29.0		28.5	Sl. curdy	28.0	Pasty	29.0		29.0		29.0		27.0	Weak
135	29.0		29.0		27.5	Mealy	28.5	Weak	29.0		28.5		28.5	Sl. crumbly
225	29.0		28.5		27.5	Mealy	28.5	Weak	28.0	Weak	28.5	Sl. open	28.5	Sl. crumbly
270	28.5	Sl. mealy	28.5		27.0	Pasty	28.5	Weak	28.5	Weak	28.5	Sl. open	28.5	Weak

[a] See table 5 for footnotes.

pasteurization of the milk, homogenization at low pressures of pasteurized milk, and addition of organic acids to milk.

The results obtained in Part I show that pasteurization of the milk used for Cheddar cheese reduced the hydrolysis of the fat in the cheese. Hydrolysis of the cheese fat, as indicated by an increase in acid number, RM, and a decrease in Polenske number, was greatest in lot 1 made from raw milk. The flavor of the cheese in lot 1 was slightly more pleasing at 9 months than that of the cheese made from pasteurized milk.

The results obtained in Part II show that homogenization of pasteurized milk at low pressures produced no significant changes in the fat constants of the cheese fat. Homogenization of the milk at 800 lb. pressure produced a weak pasty body in the cheese after aging for 9 months. A pressure of 300 lb. exerted no detrimental effect on the body.

The results obtained in Part III show that the addition of organic acids accelerated the hydrolysis of cheese fat. Acetic acid added to the cheese curd in lot 10 caused more than 300 per cent increase in acid number during aging. Other samples, along with the control lot, showed a 60 to 70 per cent increase. The quality of lot 10 was unusually low, the flavor extremely bitter, and the body weak and pasty.

The RM values for the samples were uniformly higher at the conclusion of the study than at the beginning, indicating an increase in water-soluble, volatile fatty acids during aging. The saponification numbers also tended to increase. The Polenske values, on the other hand, decreased during the same period, showing that water-insoluble, volatile acids were being reduced. The percentages of soluble acids increased and the percentages of insoluble acids tended to decrease, giving further evidence of hydrolysis of the cheese fat.

The maximum values of the total volatile acidity of the cheese samples, except lots 8 and 10, occurred at 90 days and then decreased. These two lots showed greatest total volatile acidity at 45 days.

The data on the iodine numbers are inconclusive. The variation in values from the same sample was so wide the results were not of use.

The addition of acetic and propionic acids in the quantity used produced undesirable defects in the flavor of the cheese. The lots of cheese containing citric acid and starter distillate had the highest flavor score at the conclusion of the ripening period.

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FERTILITY LEVEL IN ARTIFICIAL BREEDING ASSOCIATED WITH SEASON, HOURS OF DAYLIGHT, AND THE AGE OF CATTLE

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Under semi-feral conditions where bulls remain with the cow herd the year around, mating takes place during late spring and summer months. While animals might breed at other times of the year, there is an optimum breeding period when the reproductive powers are at a maximum.

Dairy cows under farm conditions may breed the year around, though there is a seasonal peak of calvings in the spring in most areas. In New York State dairymen complain about the difficulty experienced in getting cows with calf during winter so as to have them freshen in the fall, especially in the case of virgin heifers and old cows. Furthermore, reports have been more frequent recently of artificial breeding organizations experiencing difficulties in collecting semen of satisfactory quality from some bulls during winter.

In studies conducted by Salisbury (8) and by Mercier and Salisbury (5), data on 16 of the 40 bulls could not be used for analyses because fertility data were not available for at least 11 months during the year. The fact that of these 16 bulls 5 were young (36.6 ± 20.8 month of age) and 11 were aged bulls (94.4 ± 14.8 months of age) raised the question of whether the fertility level of bulls of extreme ages might be influenced more readily by seasons than that of bulls of medium age.

None of the publications reviewed earlier (6), nor the paper by Trimberger and Davis (11) dealing with artificial breeding, considered the possibility of differential responses of animals of varying ages to the cyclic climatic changes which occur throughout the year. The diverse results of published studies concerning the effect of season on reproduction in cattle, whether for natural or artificial breeding, do not enable one to determine if an age differential is involved. The interaction between the age of bulls and cows in determining fertility level, as reported by Tanabe and Salisbury (10), is difficult to interpret directly on physiological grounds. If an age-by-season interaction for fertility level were demonstrated, their results might be shown to be due to variations in proportion of individuals of the several age classes bred during the different seasons of the year.

Finally, if bulls of varying ages responded differently to the effect of

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climatic variations associated with the seasons of the year, the over-all fertility level in artificial breeding might be controlled more satisfactorily by use of bulls of certain ages during particular seasons of the year. The authors hoped to obtain information on which semen collection schedules could be based for bulls of various ages, in order to maintain them in fertile service longer and to obtain a maximum fertility level the year around.

The present report deals with studies on the seasonal fluctuations in fertility level of cows and of bulls of various age groups. In view of the relationship shown between hours of daylight and fertility level for Eastern Canada (6), the situation under New York conditions also was considered.

EXPERIMENTAL PROCEDURES

The data for these studies were collected from the breeding records of the New York Artificial Breeders' Cooperative, Inc., the headquarters of which are located in Ithaca, New York. The bulls were located in Ithaca, but the cows were located on 19,000 or more farms throughout the State.

Cows. The study of cows where the age was verified included 14,713 recorded services for purebred Holstein-Friesian cows bred during a 4-year period from the beginning of the association in June, 1940, until June 30, 1944. Of these services, 8,284 were services to cows bred for the first time for any single service period, 4,206 were second services or services to cows which did not conceive from the first insemination, and 2,223 were third services or services to cows which failed to conceive from a second insemination.

Registration numbers from the Holstein-Friesian Herd Books were used to determine the date of birth of each individual cow.[2] Cows arbitrarily were divided into four groups: those under 39 months, those from 39 to 63 months, those from 63 to 90 months, and those over 90 months of age. The age interval chosen was believed to correspond roughly to two consecutive conceptions in the two younger groups. The basis for this classification was the time interval found between 1,163 calvings (372 ± 46 days) of 406 cows in 12 purebred dairy herds enrolled in Dairy Herd Improvement Associations in seven New York counties. Whether or not this calving interval corresponds closely to that of purebred Holstein-Friesian cows in the State is not known, although it probably is shorter than that commonly found.

Data were sorted by months according to the age of the cows bred, regardless of the age of the bulls from which the semen was used. Monthly values were grouped into seasons, winter months being December, January, and February; spring, March, April, and May; summer, June, July, and August; and fall, September, October, and November.

The conception rate of the cows was determined indirectly by the number of successful services or the per cent of cows not coming back to service within a 5-month period after insemination. The value obtained corresponds closely to the actual percentage of conceptions as determined by the number of calves born (3) or the pregnancy diagnosis (1) and commonly is spoken of as the per cent of 5 months' non-returns.

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[2] The authors are greatly indebted to Tsuneo Tanabe for determining the age of the cows.

Bulls. The studies on bulls include 116,881 first services from 42 Holstein-Friesian and 29 Guernsey bulls used from the time the headquarters of the Cooperative were established in Ithaca, December, 1944, through Novmber, 1946. Second and third services were not considered.

The age of the bulls was determined from their birth dates and they were classified in four age groups: under 4 years of age, from 4 to 6, from 6 to 8, and over 8 years of age. The level of fertility of each male was based on the per cent of cows bred to him which were not rebred within 60 days after the first insemination, regardless of the age of these cows. The decrease in the per cent of non-returns between 2 months and 5 months is about 5 percentage units (9) and this value must be subtracted from the per cent of 2 months' non-returns to obtain a figure corresponding to the actual number of conceptions. For these studies the age of each cow was not available.

Analysis. In each study the data were grouped by months. In the case of the cows they were grouped further according to the age of the cow involved. For the bulls the data were arranged under the individual bulls and these individuals classified by age groups. For the cow data, the significance of differences between means was determined by covariance analysis using the number of cows bred as the independent variable and number of cows not returning to service as the dependent variable. This was done because in some of the age groups less than 30 cows were bred during one or another of the fall months. Cochran (2) has studied data of this type and cautions against the use of percentages where the calculated values are based on small numbers. In the data for bulls this problem was not found, for the average bull was bred to nearly 200 cows a month, so that analysis of variance of per cent non-returns was used.

RESULTS

Age and conception rate of cows. The data on conception rate of cows, including those on cows bred for the first and second time in any service period, are shown in figure 1. They indicated that the two extreme age groups fluctuated more in fertility level from season to season than did the cattle of intermediate ages. The over-all conception rate for all groups of cows was low in winter, somewhat higher in summer, and highest during spring and fall. However, in the statistical analysis of the data, the difference between the means for the four seasons was not significantly different when tested by the age × season interaction.

The age × season interaction, on the other hand, was significantly larger than was to be expected on the assumption that it was no different than the month to month variation within seasons and within the age groups. Thus, the conclusion was reached that there were marked differences between seasons in fertility level of cattle of a particular age. However, one age group performed differently from another. Whether or not a real seasonal difference in fertility level would occur in a population of cows would depend on the proportion of cows of the various ages in that population.

A study of the number of cows in each age group bred in each season showed that, in spite of a real attempt by dairymen to breed their cows for fall freshening, as cows grew older a higher proportion of them were bred in the summer. For example, among cattle less than 39 months of age, 19.3 per cent were bred in June, July, and August. For those cows over 90 months of age, 27.9 per cent were bred in those 3 months. Thus, not only does fertility level vary from season to season in a group of cows of a particular age, but so does the proportion they constitute of those cows being bred in a particular season. Roberts (7), in England, observed an increase in the proportion of spring calvings, as a consequence of an increase in summer conceptions, as cows advanced in age. This fact and practical experience support our observations.

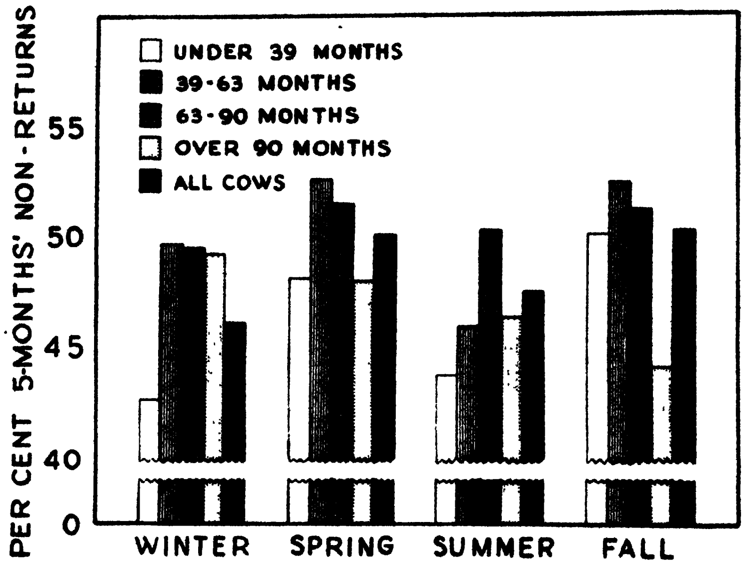

FIG. 1. Seasonal variations in fertility level of purebred Holstein-Friesian cows of different ages bred artificially in New York, June 1, 1940, to June 30, 1944.

Fertility level of bulls. The first of these studies on seasonal variations in fertility level of bulls was made on all Holstein-Friesian and Guernsey bulls used during the 2-year period, December, 1944, through November,

1946. The data are summarized for seasons and age groups in figure 2. They represent 2 months' non-returns on 116,881 first services to cows of all ages.

Analysis of variance showed that the differences in fertility level between seasons were significant at the 5 per cent level of probability when tested against the season × age interaction, which in itself was not different from the random variability in the data.

By far the heaviest use of bulls was in the 6- to 8-year-old group, which fact biased the study in the direction of determining seasonal effects within one age group. In order to obviate this difficulty, all bulls of the several age classifications which had been used consistently month after month throughout the 2-year period were selected for further study. An equal number of bulls in each age group was chosen at random from the numbers available. There were available for study three bulls in each of the four age groups, or a total of 12. A summary of the results, representing 39,833 first services to cows of all ages, is given in figure 3.

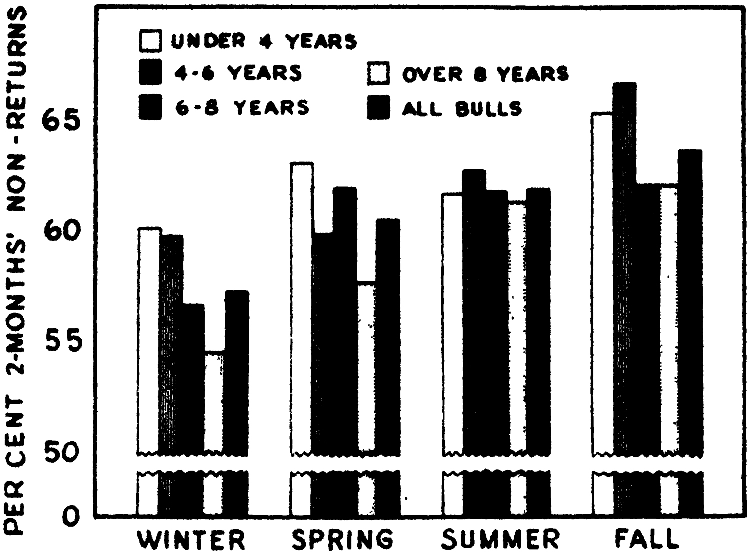

FIG. 2. Seasonal variations in fertility level of 42 Holstein-Friesian and 29 Guernsey bulls of different ages used in artificial breeding in New York at any time during the period from December, 1944, through November, 1946.

On the average, the same general trends are shown in both figures. Fertility level is the lowest in winter, gradually improving during the spring to reach a high level in the summer and fall. However, this second study, illustrated in figure 3 and orthogonal with respect to age groups, shows that each age group responded differently to season. The young bulls were relatively high in fertility in winter, gradually decreasing to a low in the hot weather of summer. Bulls in the oldest group were lowest in fertility in winter, gradually increasing in fertility level to reach a maximum in summer several weeks after the longest day of the year. Statistical analysis of these data showed a highly significant season × age group interaction when tested by the remaining variance. However, because of the size of the variance associated with this interaction, the differences observed between seasons were not found to be statistically different when tested by it.

Finally, from the total of 71 bulls used during this time interval, it was possible to select three bulls at each age interval from each of the two breeds which had been used each of the four seasons of the year, commencing in December, 1945. In some cases fertility evidence was not available each month of the year but was available for a portion of each season. This study, then, included a total of 24 bulls, 12 of each breed, and a total of 49,908 first services to cows of all ages. The data reflect an intermediate position between figure 2 and figure 3. As no significant breed by season interaction was shown, the evidence is not presented here. The fertility level of the Guernsey bulls was slightly higher than that of the Holstein-Friesian bulls, a fact which is contrary to the observations noted earlier (5).

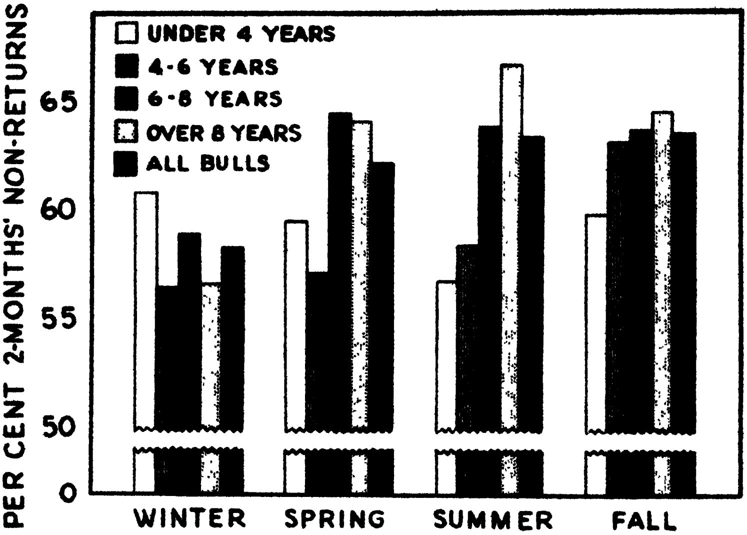

FIG. 3. Seasonal variations in fertility level of three bulls in each of four age groups used in artificial breeding in New York consistently month by month during the period from December, 1944, through November, 1946.

It is of interest to note that proper temperature control of the semen during the summers of 1945 and 1946, by use of the semen carrying chest, eliminated the low fertility results during summer observed earlier and shown in figure 1.

Hours of daylight and fertility level. In view of the relationship between the hours of daylight and the monthly level of fertility observed earlier for Canadian conditions (6), correlation coefficients between these items for groups of bulls of various ages were determined. The records used were from December, 1945, through November, 1946, and include data on five bulls in each age group used regularly each month of the year, as well as data on all bulls used throughout the year. In order to get an idea of the length of the apparent lag effect of daylight variations, correlation coefficients between fertility level for one month and the average length of daylight for the month corresponding to and for the first, second, and for the third month prior to service were determined. The correlation coefficients are presented in table 1. No significant correlation between these two variables was observed for bulls under 6 years of age. It was high for bulls of 6 to 8 and 8 to 10 years of age, low for bulls over 10 years of age, and high for all bulls. The correlation between fertility level and length of daylight, on the whole, was highest for the first and second month prior to service.

TABLE 1

Correlation coefficients between monthly fertility level of bulls of various ages and the length of daylight in Ithaca, N. Y.

Age of bulls (yr.)	Months for length of daylight							
	Corresponding		Prior to services					
			First		Second		Third	
	5 Bulls	All	5 Bulls	All	5 Bulls	All	5 Bulls	All
Under 4	0.29	0.38	0.30	0.35	0.22	0.22	0.05	0.00
4–6	0.06	0.16	0.25	0.36	0.40	0.48	0.44	0.49
6–8	0.36	0.27	0.65*	0.56*	0.75†	0.69†	0.64*	0.63*
8–10	0.47	0.64*	0.47	0.71†	0.30	0.56*	0.08	0.28
Over 10	0.45	0.66*	0.56*	0.68*	0.52	0.53	0.33	0.25
All bulls	0.48	0.56*	0.67*	0.75†	0.67*	0.73†	0.49	0.51

* Significant at the 5% level of probability.
† Significant at the 1% level of probability.

The over-all relationship between these two variables under Canadian and New York conditions agrees closely. The Canadian results involve records from three herds for 25 years on cattle of all ages bred normally. The New York results involve records collected during 1 year from 19,000 or more farms where cattle of all ages were bred artificially.

DISCUSSION

Because of the enormity of the task, it was impossible to establish the age of both the bull and the cow for each service involved in these studies. Such data would enable one to determine the possible seasonal interactions in fertility of mates of the same and of different ages. The fertility interaction between the various ages of bulls and cows, as observed by Tanabe and Salisbury (10), might be explained on that basis.

Also, it would have been desirable to have the same uniform schedule of semen collection for all bulls involved in these studies. However, this was impractical because of the limited number of bulls available and of the seasonal demand for semen. No significant relationship between fertility and the same general frequency of semen collection was observed earlier under New York conditions (5).

Nevertheless, the results obtained in this investigation show that with respect to fertility level, males and females of various ages, regardless of the age of their mates, responded differently and consistently to the seasons of the year. The fertility level of younger and older cattle fluctuated more from season to season than that of cattle of medium age.

Winter was the poorest breeding season of the year for all age groups at this latitude. The fact that one-third of the yearly number of artificial services in New York occurs during winter when the fertility level of cattle is at its minimum creates a serious problem. This management practice decreases the over-all fertility level. One way to improve this condition would be to breed young cows during early spring and fall, cows of medium age during winter, and old cows during late spring and summer, if this practice did not upset the seasonal distribution of calvings desired. Young bulls probably should be used least during the summer, and may be used advantageously during the winter, when the fertility of older bulls is at a low level.

In an earlier publication (5) the authors obtained results dealing with the semen production and fertility level of a group of relatively young bulls which showed the poorest fertility level in the summer, in spite of the fact that the temperature of the semen was maintained properly. Those results would be expected on the basis of the results obtained here with bulls of equivalent age. However, if only the average results for all bulls in each case are compared, the fertility levels shown for the seasons are quite different. In their earlier review of the literature (5), the present authors stated that there was a lack of unanimity among investigators concerning effect of season on reproductive performance in bulls. The present results, showing an interaction of season and age of bulls, resolve the disagreement, and the earlier studies by others support the picture as described.

As discussed earlier (6), the authors believe that light influences reproductive functions in cattle by its action on the pituitary through the eye and the optic nerve. The different reproductive responses of cattle of various ages to seasons are believed to be due to a varying priority need for pituitary substances by various physiological functions of the body. This theory does not exclude the possibility that other endocrine organs subordinated to the pituitary might be involved in the differential response of cattle of various ages to the season of the year. Work needs to be done to demonstrate the mechanism of light on fertility level of cattle of various ages, and to test the possibility of using artificial light to improve the fertility level of cattle bred during winter.

SUMMARY

In an investigation on the seasonal variations in fertility level of about 125,000 cows and 71 bulls of various ages bred artificially within the last 6 years in New York State, it was found that:

1. With respect to fertility level, cattle of various ages responded differently and consistently to seasons of the year, the younger and older cattle being influenced more readily than mature cattle.
2. Winter was the poorest breeding season of the year during this period.
3. The fertility level of all bulls kept at this Station but artificially bred to cattle in all sections of the State from December, 1945, through November, 1946, was significantly correlated with the length of daylight, there being a lag of 1 or 2 months before the effect of daylight reached its maximum. For two groups of bulls less than 6 years of age, the correlation coefficients were not statistically significant. Thus, young bulls probably are more subject to other extraneous influences on fertility than are those from 6 to 10 years of age.

It is concluded that variations in the hours of daylight influence the fertility level of dairy cattle at this latitude, and that the response to light varies with the age of the animals involved.

The authors are grateful to R. W. Bratton for his help in preparing the manuscript.

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RATING THE ACID-PRODUCING ABILITY OF STARTERS FOR CHEDDAR CHEESEMAKING[1]

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The purpose of adding starter to milk in the manufacture of Cheddar cheese is to induce the development of lactic acid at a dependable rate and to secure thereby a maximum measure of control of the vat. To achieve this end, a knowledge of the behavior of the starter *per se* is fundamental.

Whitehead and Cox (9) developed a method of determining the vitality of starters which consists of simulating, in miniature, the various steps in the cheese vat. The increase in the titratable acidity of whey between the fifth and sixth hours is taken as the vitality of a starter relative to another starter treated in the same way. Babel (1) used the acidity attained at intervals of 1 or 2 hours during 7 hours under specified conditions as an index of the acid-producing ability of starter cultures.

A somewhat more fundamental approach, with definite advantages, can be made to the problem of rating the acid-producing ability of starters. The lactic acid-producing property of starter organisms basically is the effect of one or more enzyme systems. It is logical to assume that under defined conditions rate of acid production should be characteristic of that starter.

Before proceeding further, a clear distinction should be made between the scientific use of the word *rate* and its somewhat broader meaning. For instance, in their review of butter starters, Hammer and Babel (7) state that ". . . the rate of a ripening culture is controlled primarily by varying the amount of inoculum . . .". Similarly, in his bulletin, Hales (6) states that ". . . the amount of inoculation affected the rate of acid development . . .". These and other authors refer to a static concept implying the over-all rate or the time required for a culture to reach a maximum acidity.

When used more precisely, the word *rate* is a dynamic concept. This usage may be illustrated by referring to a discussion of the growth phases of bacteria in any standard book on bacteriology. The rate of growth keeps changing from a stationary, through accelerating, maximum, decelerating, and, finally, stationary phases. Graphically it is indicated as the slope of the curve.

According to this second concept, the data of Monrad and Helmer, reproduced by Hales (6), show that the rate of lactic acid production measured as titratable acidity is independent of the amount of inoculum used. All the curves shown are practically identical in shape but are merely displaced

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on the time axis. Similarly, Dolby (4) showed that the rate of lactic acid development in the cheese curd, measured as pH, is independent of the amount of starter used.

In order to characterize a starter by its rate of lactic acid production, it is necessary to establish a relationship between acid development and time. It was indicated by Baker *et al.* (2) that the rate of lactic acid development, whether measured as titratable acidity corrected for the apparent acidity of milk or as pH, tended to be in geometric progression.

In this communication evidence is submitted to show that under defined conditions the titratable acidity of a milk culture as ordinarily determined (*i.e.*, uncorrected) increases according to the geometric series with reasonable accuracy. This relationship then is used to develop a method of rating cheese starters according to their acid-producing ability in which a constant called the starter quotient, characteristic for each starter, is derived from titratable acidities.

EXPERIMENTAL

Principle. The method employed in this study depends upon the observation that in a milk culture under defined conditions titratable acidity increases in geometric progression with time, which increases in arithmetic progression over the major part of the range over which lactic acid is produced. Mathematically, this relationship between titratable acidity and time in its simplest form can be expressed by the equation:

$$a^{1/t} = r \qquad \text{(a)}$$

where a = titratable acidity
t = time at which a is determined
r = a constant and is equal to the ratio of the two adjacent members in the geometric progression.

In practice, however, it is more convenient to use the expression

$$\frac{\text{Titratable acidity at time } t_1}{\text{Titratable acidity at time } t_2} = k \qquad \text{(b)}$$

where k is a constant for a fixed time interval $t_2 - t_1$.

Method. All starters used in these studies were carried in skim milk autoclaved at 10 lb. pressure (115.5° C.) for 20 minutes. Transfers were made twice a week. A 1 per cent inoculum was propagated at 72° F. for the first 17 hours, and the starter then was stored in a refrigerator at 40° F. until the next transfer.

For the determination of the lactic acid-producing ability, starter transferred on a previous day is used. It is inoculated at the rate of 3 per cent (7.5 ml.) into 250 ml. pasteurized whole milk at 98° F., contained in a half-pint (Imperial measure) bottle fitted with a no. 8 rubber stopper. The milk then is mixed thoroughly, the time recorded, and a 9-ml. portion is titrated with 0.100 N sodium hydroxide, using phenolphthalein as indicator. The bottle then is placed in a suitable water bath at 98° F. It is convenient to

TABLE 1

Derivation of starter quotients from titratable acidities of starter cultures

Starter no.		Time in hours												
		0		1		2		3		4		5		6
43	Titratable acidity (%)	0.17		0.19		0.22		0.29		0.40		0.53		0.61
	1-hr. quotient						76		**73***		**76**		**87**	
	2-hr. quotient							55		**55**				
CD	Titratable acidity (%)	0.17		0.19		0.22		0.27		0.34		0.45		0.56
	1-hr. quotient						82		**79**		**76**		**80**	
	2-hr. quotient									**60**		**61**		
SH9	Titratable acidity (%)	0.17		0.18		0.21		0.25		0.30		0.35		0.42
	1-hr. quotient						84		**83**		**86**		**83**	
	2-hr. quotient									**72**		**71**		
SH17	Titratable acidity (%)	0.16		0.17		0.19		0.22		0.25		0.29		0.33
	1-hr. quotient						86		87		**86**		**88**	
	2-hr. quotient									76		**76**		
KAS	Titratable acidity (%)	0.17		0.19		0.23		0.32		0.45		0.58		.....
	1-hr. quotient				83		**72**		**71**		77			
	2-hr. quotient							**51**						
EDM	Titratable acidity (%)	0.17		0.18		0.22		0.31		0.46		0.62		.....
	1-hr. quotient						71		**67**		74			
	2-hr. quotient							48		50				

* Values in bold face indicate those which meet the requirements of the defined quotient.

start the determination on the hour or the half-hour. Starting with the initial titration as zero hour, the milk is titrated at 1, 2, 3, 4, and 5 hours and recorded. It also must be mixed by inverting the bottle at the half-hour as well as at the hour. This is necessary to prevent the accumulation of bacteria in the upper layer of the milk due to creaming.

The lactic acid-producing ability of the starter now is calculated by dividing the first titratable acidity which is 0.23 per cent or over by the titratable acidity obtained 2 hours later, provided that the second titration does not exceed 0.56 per cent as lactic acid. The result is expressed as a simple ratio, omitting the decimal point for convenience, and is termed the starter quotient.

Properties of the starter quotient. The first important property of the starter quotient which must be established is its constancy. Representative data are presented in table 1. Corresponding to each starter, the first line gives the titratable acidities obtained by the method already described, at the hours indicated. The second lines gives the 1-hour starter quotients derived from the titratable acidities 1 hour apart. The last line gives the 2-hour quotients derived from the appropriate values 2 hours apart in the first line.

TABLE 2

Reproducibility of the starter quotient

Starter 43		Starter CD	
Date	Starter quotient	Date	Starter quotient
Jan. 21	52	Jan. 21	63
Jan. 22	53	Jan. 22	62
Jan. 23	54	Jan. 23	61
Feb. 6	55	Feb. 4	56
Feb. 12	53	Feb. 27	55
Feb. 14	55	March 13	51

The following observations can be made by studying table 1. Both the 1-hour and the 2-hour starter quotients remain constant for a given starter regardless of the values of the two titratable acidities used for their calculation or the hours at which these determinations are made. The useful range of titratable acidities over which a constant starter quotient may be obtained is from 0.23 to 0.56 per cent as lactic acid. These relationships hold for the more active starters such as 43, EDM and KAS, as well as for the slow starters such as SH17 and SH9. In order to include a large portion of the range from 0.23 to 0.56 per cent titratable acidity and perhaps to submerge some of the slight irregularities which appear in the 1-hour quotient, the 2-hour starter quotient has been adopted as standard.

The next important question to be answered is the reproducibility of the results. Illustrative data on starters 43 and CD are summarized in table 2.

Starter 43 has been used in experimental cheese work in these laboratories and also is supplied from time to time to those requesting a cheese starter. It is carried under standardized laboratory conditions. On the whole, the starter quotient for 43 has remained unchanged over even a longer period than that shown in table 2. Starter CD was obtained from a commercial dairy, where it occasionally was used as a buttermilk starter but had not been given much care. It was selected for study because of its poor condition. It can be seen from table 2 that on consecutive days the value of the starter quotient showed satisfactory reproducibility. Over a period of 6 weeks much improvement or recovery was shown by this starter under proper care. The ability of some starters to improve is known and in the above instance was expected.

In general, the reproducibility of the starter quotient is considered satisfactory, keeping in mind the biological basis of the determination and excluding the long-term changes in the properties of starters, which really form a part of another problem.

A further characteristic property of the starter quotient is its independence of the percentage of the starter inoculum used in the milk culture. In table 3 are presented data obtained when starter 43 was inoculated at the rate of 1, 2, 3, and 4 per cent and the determination of the starter quotients carried out as described. The starter quotients, whether calculated on an hour or a 2-hour basis, show good agreement for all the levels of inoculum employed.

In summarizing the properties of the starter quotient, it may be said that it is a specific index of the acid-producing ability of the starter.

Comparative rating of starters. While no doubt there are excellent starters without pedigree used in the manufacture of Cheddar cheese, in general, they are derived from several central sources of supply. Available starters were obtained from St. Hyacinthe Dairy School, Kemptville Agricultural School, the Dairy Department of the Ontario Agricultural College, and the Dairy Department of the University of Alberta, all of which supply starters to the Cheddar cheese industry. These, together with the Dairy Research starter and one from a commercial dairy (CD), are included in table 4 in a comparative rating of their acid-producing ability by means of their starter quotients.

Each starter was transferred once when received and the starter quotient determined the next day. This is shown as the initial starter quotient in the table. The starters then were carried for 3 to 15 weeks under conditions referred to earlier; a final starter quotient at the end of the period indicated also is given in the table. In addition, starter quotients calculated from Babel's data (1) for titratable acidities at the fifth and seventh hours, with incubation at 98° F., are included for comparison. These are starters B1 to B5, and B7, the original numbers of the author being retained. Some

TABLE 3

Constancy of the starter quotient at varying percentages of inoculum (Starter 43)

Per cent inoculum		Time in hours										
		0	1	2		3		4		5		6
1	Titratable acidity (%)	0.17	0.17	0.19		0.20		0.26		0.37		0.49
	1-hr. quotient								**70***		**75**	
	2-hr. quotient									**53**		
2	Titratable acidity (%)	0.17	0.18	0.20		0.26		0.36		0.49		. . . .
	1-hr. quotient						**72**		**73**			
	2-hr. quotient							**53**				
3	Titratable acidity (%)	0.18	0.19	0.23		0.30		0.43		0.58		....
	1-hr. quotient				**77**		70		74			
	2-hr. quotient					**54**		52				
4	Titratable acidity (%)	0.18	0.20	0.25		0.35		0.48		. . . .		. ..
	1-hr. quotient				**72**		**73**					
	2-hr. quotient					**52**						

* Values in bold face indicate those which meet the requirements of the defined quotient.

starters were omitted because they did not provide titratable acidities 2 hours apart in the range 0.23 to 0.56 per cent.

Starter quotients for the 19 starters listed indicate widely varying abilities to produce lactic acid. The Canadian starters and Babel's starters are listed in separate series, in order of their acid-producing ability. The starters listed first in table 4 are the most active and would be expected to be the most satisfactory for Cheddar cheesemaking. As a matter of experience, starter 43 with a starter quotient of 52 has been found very satisfactory in making cheese over a period of several years.

For the majority of starters, the difference between the initial and the final ratings after 3 to 15 weeks is not large. In other words, with the exception of starters OACNZ and CD, a stability of the acid-producing ability is indicated.

TABLE 4

Comparative rating of starters

Starter no.	Initial starter quotient	Final starter quotient	Interval (weeks)	Starter no.	Initial starter quotient	Final starter quotient	Interval (weeks)
SH4	47	50	3	SH9	71	71	3
SH5	47	50	3	SH16	74	68	3
EDM	48	54	3	SH17	76	71	3
KAS	51	51	9				
43	52	52	15	B3*	55		
OACNZ	55	66	4	B2	59	...	....
CD	63	53	10	B4	60		.
SH29	63	67	3	B1	65		.
OACH	64	70	4	B7	66	..	..
SH6	67	68	3	B5	78	..	......

* Data on B-series calculated from data of Babel (1).

DISCUSSION

In developing the procedure which finally was adopted for the determination of the starter quotient, consideration was given to those factors which would give a simple yet useful method for the evaluation of starters used in Cheddar cheesemaking. The equipment used is either ordinarily available or readily obtainable. Pasteurized milk was adopted as the medium in the test because a more uniform product of good bacteriological quality is more readily available at all times. Impairment of the acid-producing ability of some starters at cooking temperatures in cheesemaking and of most starters at slightly higher temperatures has been reported by several investigators (1, 3, 4, 5, 8, 10, 11). In fact, high cooking temperature is a standard method of retarding too rapid acid development in the vat. For this reason and because a good starter must be able to withstand at least this temperature during cooking, 98° F. was selected for the determination of the starter quotient.

With respect to the starter quotient itself, it already has been pointed out that it is constant over the major portion of the range over which lactic acid is produced, and is characteristic of a given starter. Furthermore, it is independent of the amount of inoculum used and allows a considerable latitude with respect to the choice of time or titratable acidities which may be used for its calculation. This makes it possible to make comparisons on an absolute basis of starter quotients determined in different laboratories under various conditions. A 3 per cent starter inoculum has been selected for our determination since it allows a determination to be carried out conveniently during the working day, and still gives good results. However, should it be necessary, a different percentage of inoculum may be used. The starter quotient is independent of the initial stationary and lag phases and the final inactive and death phases of bacterial growth. Much of the uncertainty which characterizes these phases and is included in a determination of the over-all growth rate therefore is eliminated from the starter quotient. Finally, from a comparison of acidity-time curves such as those of Monrad and Helmer (6) for milk and the ones given by Dolby (4) for cheese curd, it appears that the rates of acid development in milk and in cheese curd are not dissimilar. To this extent, therefore, a rating of a starter in milk culture also should be an index of its behavior in the cheese curd.

Only brief comment is offered on the question of variability of the starter quotient. The cheese starters in common use on this continent are made up of several species of lactic acid-producing organisms. An equilibrium among these organisms is maintained by uniformity of the conditions in carrying the starter. In addition, there is a variability of properties of a normal population of each species or each strain. Equilibria between the acid tolerant and non-tolerant organisms and between temperature tolerant and non-tolerant organisms would be important. The starter quotient, therefore, is an index of the average acid-producing ability of all the organisms present in the starter. While a starter may, over a period of time, become unsuitable for cheesemaking from the point of view of acid production, this is not a weakness of the starter quotient; on the contrary, the starter quotient may be used in ascertaining when the starter needs attention or replacement.

SUMMARY

Starter which has been transferred the day before is inoculated at the rate of 3 per cent into 250 ml. pasteurized whole milk and incubated at 98° F. Titrations are made at 0, 1, 2, 3, 4, and 5 hours, and, in addition, the milk cultures are mixed every half hour. The lactic acid-producing ability of the starter is calculated by dividing the first titratable acidity which is 0.23 per cent or over by the titratable acidity obtained 2 hours later, provided that the second titration does not exceed 0.56 per cent. The result is expressed as a simple ratio, omitting the decimal point for convenience, and

is termed the starter quotient. Mathematically, the starter quotient is based upon the observation that titratable acidity in a milk culture under defined conditions increases geometrically. The quotient remains constant over the range 0.23 to 0.56 per cent and is independent of the amount of inoculum used. Data are presented illustrating the properties of the starter quotient, and a comparative rating of 13 Canadian cheese starters is given.

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OBSERVATIONS ON THE INSTABILITY OF THE PROTEIN PHASE OF FROZEN CONCENTRATED MILK[1]

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The gradual progressive flocculation of some of the protein of milk as a result of frozen storage has been observed by a number of workers, some of whom (1, 2, 3, 5, 6, 8, 9, 11, 14) have noted that the instability of the protein phase is aggravated by such influences as lengthened periods of storage, comparatively high storage temperatures, increased concentration of the milk, and comparatively high heat treatments applied as forewarming or superheating procedures in advance of freezing. It also has been noted that the flocculation is reversible with heat to a remarkable degree (5, 8, 9, 14), but factors affecting reversibility have not been studied. Little is known concerning the effect of "stabilizing salts" or of higher pH in delaying this type of protein precipitation (4, 10, 11), although with other types of coagulation they are known to be inhibiting.

The causes of protein flocculation in frozen stored milk are not established definitely, but they are best postulated by Sommer (13), who suggests that higher salt concentration, probable higher acidity, physical crowding of the protein micelles by ice crystals, and loss of water of hydration are the destabilizing factors, with the increasing viscosity as an opposing factor. Webb and Hall (14) are convinced that calcium caseinate (a weakly hydrated sol in milk) comprises the bulk of the flocculated protein and believe that frozen storage further lessens the hydrophylic character of this protein so that on thawing it behaves more as a strictly hydrophobic substance.

Existing knowledge concerning the flocculation phenomenon still is meager and in several respects inadequate. In view of the likelihood that more milk and milk products will be stored and handled in the frozen state as the possibilities become better appreciated, it seemed desirable to acquire more information concerning the instability of the protein phase, particularly since this appears to be the limiting factor in the storage life of frozen concentrated forms of milk.

EXPERIMENTAL METHODS

Inasmuch as protein flocculation during frozen storage was the only phenomenon under study, all observations were made on skimmed milk to eliminate the interfering effect of the fat phase. Fresh, factory-skimmed milk was forewarmed, concentrated, cooled, standardized, filled into suitable

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pint containers, and frozen statically at approximately – 10° F. (– 23.3° C.). After 24 hours, when solidly frozen, it was stored at either + 5° F. (– 15° C.) or at – 10 to – 15° F. (– 23.3 to – 26.1° C.). A sufficient number of pint containers of each lot of milk was filled to allow periodic withdrawal of one at each observation during the course of frozen storage. The higher storage temperature used was obtained in a large, accurately controlled ice cream cabinet, and the lower in an experimental hardening room which fluctuated about 5° F. (2.8° C.), although in a few comparisons the fluctuation amounted to 10° F. (5.6° C.).

When examined, frozen samples were taken from the containers, weighed into 2-l. beakers and reconstituted and thawed with the proper weight of distilled water at 180° F. (82.2° C.). Defrosting was accomplished without agitation and the appearance of the thawed samples was noted. They then were thoroughly agitated by pouring ten times.

Volume of Flocculated Protein

A 50-ml. portion of the well-mixed sample was placed in a graduated, tapered, centrifuge tube and whirled at 2,000 r.p.m. for 15 minutes in a centrifuge with a 12-inch head. The tubes were decanted carefully, made up to the original volume with ice-cold distilled water, centrifuged again for 10 minutes, and the per cent of flocculated protein measured by volume. This method is similar to that of Anderson and Pierce (1), except that they weighed the protein in a wet state, and is almost identical with that of Babcock *et al.* (2), who reported results as "cc. of sediment".

Other Tests

Alcohol numbers were determined as described by Doan (7). Titratable acidity measurements followed the recommendation of Sommer (12). Determinations of pH were made at 25° C. with a Beckman industrial model pH meter, utilizing a glass electrode standardized by means of buffer tablets made by Coleman Instrument, Inc., according to the formulas of Kolthoff, McIlvaine, and others, using sodium tetraborate and potassium phosphate.

Heat Redispersion of Flocculated Protein

About 100 ml. of a well-mixed defrosted and reconstituted sample were placed in a 300-ml. beaker and heated over a low flame with continuous agitation until a thermometer in the liquid registered exactly 150° F. (65.5° C.) or 200° F. (93.3° C.), depending on the degree of flocculation present, after which the beaker immediately was immersed in ice water. After heat treatment, the volume of flocculated protein again was determined as a measure of the degree of redispersion accomplished by the heat treatment.

Other methods and variations employed are described in the appropriate places.

EXPERIMENTAL RESULTS

The appearance of variously treated samples of concentrated skim milk after 12 weeks of frozen storage is illustrated in figure 1. These samples were defrosted and reconstituted without agitation. They exhibit various abilities to disperse into the water of reconstitution, depending on the individual degree of protein denaturation. Figure 2 shows two of the samples, of differing dispersibilities, after pouring ten times and standing 10 minutes. It will be noted that sample no. 2 presented an entirely normal appearance after agitation, whereas no. 4 showed a definite tendency to settle on the part of the small flakes present. When the no. 4 sample was heated to 150° F., however, it became just as normal in appearance as no. 2.

FIG. 1. A series of samples of thawed and reconstituted frozen concentrated skim milk after 12 weeks storage. (Samples 1 to 4, forewarmed at 190° F. for 15 minutes; 5 to 8 at 190° F., flash. Samples 1, 2, 5, and 6 stored at – 10 to – 15° F.; 3, 4, 7, and 8 at + 5° F. Samples 1, 3, 5, and 7 reconstituted with water at 80° F.; 2, 4, 6, and 8 with water at 180° F.)

Nature of the Floc

Characteristics. The protein flocculation gradually occurring during the frozen storage of concentrated milk is not apparent while the milk is frozen. On thawing, and particularly on thawing accompanied by dilution with water, it first becomes evident as a fine, feathery, and somewhat fragile precipitate. Initially, it seems to redisperse on mere standing. When more pronounced, it can be dispersed by vigorous agitation, but eventually denaturation proceeds so far that no amount of simple agitation will cause it to assume a normal dispersed condition. In advanced stages, a gross precipitation and a wheying-off are evident.

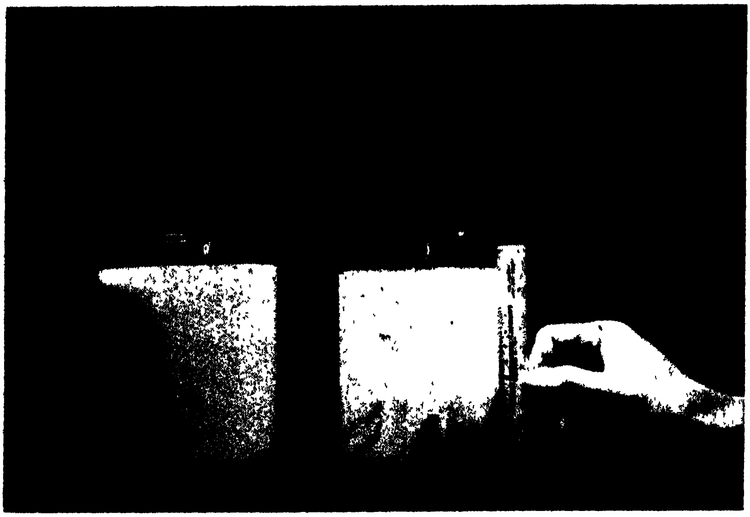

FIG. 2. Two of the samples shown in figure 1 after pouring ten times and standing 10 minutes. (Sample 2 stored at – 10 to – 15° F. Sample 4 stored at + 5° F.)

Freezing alone causes no measurable precipitation of the protein. This was noted scores of times during the course of the study. Measurable amounts of floc were obtained only after a time interval in the frozen state, the length of the interval being governed by other conditions. Typical results are shown in table 1. No significant deposits of protein were obtained on centrifugation of these samples until after 1 week of storage at + 5° F., or until after 8 weeks of storage at – 10 to – 15° F.

The relative fragility of the floc and its tendency to redisperse in the

TABLE 1

The volume of flocculated protein produced by frozen storage of concentrated skim milk (4:1)

(Reconstituted basis)

Weeks in storage	Immediately after thawing and mixing		2 hr. at room temperature after thawing and mixing	
	At – 10 to – 15° F.	At + 5° F.	At – 10 to – 15° F.	At + 5° F.
	(%)	(%)	(%)	(%)
Unfrozen	0.50	0.50	0.50	0.50
0	0.50	0.50	0.50	0.50
1	0.50	8.00	0.50	2.50
2	0.50	15.00	0.50	7.00
4	0.75	15.50	0.50	15.00
8	1.00*	15.00	0.75	16.00
10	2.50	15.00	0.75	18.00
12	8.00	15.00	0.75	17.50

* Visual evidence of flocculation in thawed reconstituted samples is noted between 1.00 and 2.00 per cent.

early stages are well shown by a comparison of the volumes obtained immediately after thawing and after 2 hours of standing. In the later stages, redispersion is not evident; in fact, the tendency is toward greater volume of precipitate, which may be due to gradual hydration insufficient to cause peptization but enough to increase the bulk of the deposit in the centrifuge tube. It is believed that this tendency to redisperse on standing is responsible for some of the minor fluctuations noted in the data on volume of precipitate throughout the study.

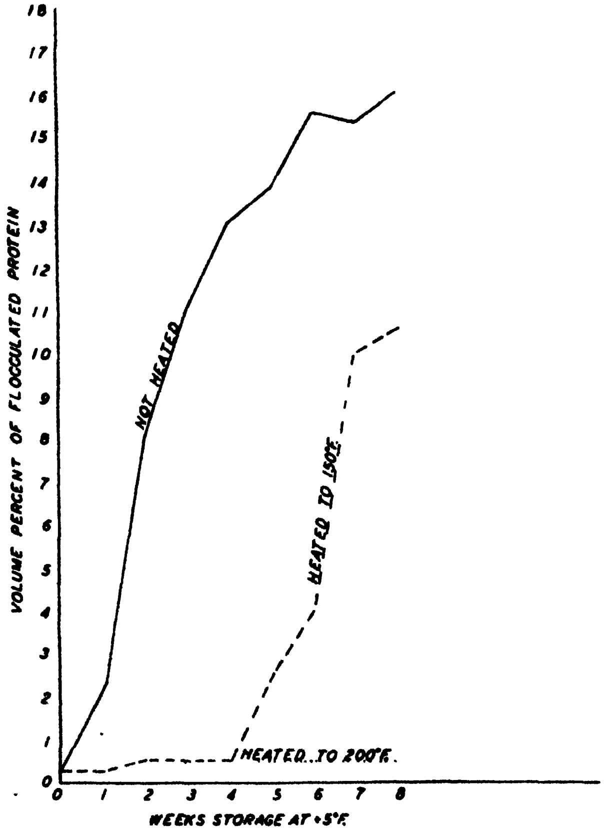

FIG. 3. Redispersion by heat of flocculated protein in frozen and stored concentrated skim milk (3:1) (average of 13 trials).

Reversibility with heat and agitation. The effect of heat in peptizing the flocculated protein of thawed and reconstituted frozen milk after various intervals of frozen storage is strikingly shown in figure 3. After 8 weeks of storage, the samples exhibited an average of about 16 per cent flocculation. When heated to 150° F., they showed only about 10 per cent and when heated to 200° F., the volume of flocculation was insignificant. Agitation and homogenization alone or coupled with heat also causes a redispersion of frozen storage flocculated protein, as shown by the data in table 2. It appears, therefore, that the redispersion of this protein is in the nature of a simple dissociation accomplishable by heat or mechanical work.

Similarity to "salted-out casein". Observations on the protein flocculation encountered in this study led to the belief that the precipitation is in the nature of a "salting out" process, readily reversible in the early stages merely by removing the cause but becoming less reversible with time. The effect of the time factor on the properties of a "salted-out" calcium caseinate held in the brine at room temperature was compared with the properties previously presented of a protein flocculated under conditions of frozen storage.

TABLE 2

Redispersion of protein flocculated in milk by frozen storage as influenced by heat and mechanical work

Treatment of thawed reconstituted milk	Volume of flocculated protein	
	Stored 4 weeks	Stored 8 weeks
	(%)	(%)
Immediately after mixing	13.00	16.50
Standing 2 hr. after mixing	9.00	18.00
After mixing and heating to 150° F.	1.00	10.50
After mixing and heating to 200° F.	0.75	3.00
After mixing and homogenizing* at 70° F.	4.00	11.50
After mixing and homogenizing* at 150° F.	0.50	1.50
After mixing and homogenizing* at 200° F.	0.50	0.75
After mixing and agitating† at 70° F.	3.00	10.00
After mixing and agitating† at 150° F.	0.75	1.00
After mixing and agitating† at 200° F.	0.50	1.00

* Accomplished by means of a laboratory hand homogenizer.

† Accomplished with a Waring Blendor in 60 seconds.

A portion of concentrated skim milk (3:1) was treated in a large Erlenmeyer flask with 36 g. of sodium chloride per 100 g. of water present and allowed to stand at room temperature. At intervals the precipitated mixture was agitated thoroughly and two samples removed for examination. The first was simply reconstituted, resulting in a dispersion which was about one-fourth saturated with sodium chloride. A portion of this was allowed to stand at room temperature for 2 hours while two other portions were heated to 150 and 200° F., respectively. The second sample was dialyzed against cold tap water for 48 hours followed by reconstitution, and a portion of this was heated to 200° F. The volume of the precipitate then was determined on all portions of each of the samples in the usual manner. The results are given in table 3.

The development and behavior of salted-out calcium caseinate stored in the brine and of protein precipitated from milk by frozen storage are, to all intents and purposes, identical. At first, both are reversible with removal of the cause, but upon introduction of a time factor, reversibility gradually is lost. Both are reversible for a time by heating, but eventually this too becomes ineffective.

Some of the dialyzed calcium caseinate salted from skim milk was compared with the washed coagulum obtained from some of the frozen and stored concentrated milk samples after dispersing by reconstituting and heating. Treatment with acids, alkalies, salts of heavy metals and alcohol, saturation with sodium chloride and magnesium sulfate, heating to boiling, and additions of rennin and rennin plus calcium chloride gave identical results. The sols from each type of precipitate gave similar isoelectric ranges (pH 4.60 to 4.72), as judged by the turbidity of filtrates from series tests made by adding increasing amounts of acetic acid. It seems justifiable to conclude that the flocculation developing in frozen and stored milk is calcium caseinate, as Webb and Hall (14) have assumed. Only traces of albuminous substances were detectable in the prepared sols from frozen milk flocs, indicating that these were in the nature of contaminants and not a part of the coagulum proper.

TABLE 3

Volume and behavior of salted-out calcium caseinate when stored in the brine at room temperature in the form of concentrated skim milk (3:1) saturated with sodium chloride

Stored at room temperature	Volume of flocculated protein					
	Undialyzed but reconstituted (about ⅓ saturated with NaCl)				Dialyzed 48 hr. and reconstituted	
	At once	After 2 hr. standing	Heated—150° F.	Heated—200° F.	Cold	Heated—200° F.
(*weeks*)	(%)	(%)	(%)	(%)	(%)	(%)
0	0.25					
1	3.00	0.50	0.25	0.25	0.25	
2	4.00	2.00	0.25	0.25	0.25	
4	7.50	3.50	0.25	0.25	0.25	
6	10.00	8.00	1.00	0.25	0.25	
8	22.00	10.00	2.50	0.75	1.00	0.25
12	24.00	18.00	7.50	3.00	26.00	2.00
14	24.00	26.00	20.00	17.00	28.00	3.00
18	22.00	23.00	24.00	22.00	34.00	6.00
22	24.00	26.00	24.00	24.00	31.00	24.00

The Effect of Freezing and Frozen Storage of Concentrated Skim Milk on Titratable Acidity, pH, and Alcohol Number

Determinations of titratable acidity, pH, and alcohol number (protein stability) were made on a large number of skim milk samples variously treated, concentrated, frozen, and stored. These were made before freezing, after freezing, and at various intervals during frozen storage. No significant changes in any of these values with any of the samples were noted when comparisons were made on an equivalent reconstituted basis. Alcohol numbers, of course, could not be obtained on stored samples which exhibited visible flocculation. Nevertheless, when the protein of such samples was redispersed by heating, the alcohol number showed no change from the original unfrozen sample. It therefore can be concluded that neither freezing nor freezing and storage causes any permanent change in these three characteristics of milk.

Effect of Forewarming Temperature of the Concentrated Skim Milk on Protein Stability in Frozen Storage

Preliminary comparisons showed that the interval of heating fluid skim milk prior to condensing exercised an insignificant influence on the stability of the protein in subsequent frozen storage but that the temperature used had considerable effect. Several trials were made using forewarming temperatures from 145 to 208° F. (62.8 to 97.7° C.). Typical results are presented in table 4 for samples stored at −5 to −15° F. Similar results were obtained at +5° F., but the flocculation was much more rapid and the effect of the variable was less clear.

TABLE 4

The volume of flocculated protein in frozen and stored concentrated skim milk (3:1) as influenced by the forewarming temperature

(Storage at −5 to −15° F.)

Weeks in storage	Temperature of forewarming (approx. 15 min.)					
	145° F.	160° F.	170° F.	180° F.	190° F.	200° F.
	(%)	(%)	(%)	(%)	(%)	(%)
Unfrozen	0.50	0.50	0.50	0.50	0.50	0.50
0	0.50	0.50	0.50	0.50	0.50	0.50
2	0.50	0.50	0.50	0.75	0.50	0.75
4	0.50	0.50	0.50	0.75	0.75	0.75
6	1.00	0.50	0.50	1.00	0.75	0.75
8	1.00	0.75	0.50	1.50	2.00	1.50
10	1.00	0.75	0.75	5.25	5.50	6.50
12	1.25	0.75	0.75	11.00	7.25	9.00
14	1.25	1.00	0.75	11.50	9.00	13.75
16	1.50	1.00	0.75	13.50	12.50	13.75

Temperatures of forewarming between 145° F. and the apparently critical point of 170° F. do not influence materially the rate of flocculation, but with higher temperatures the stability of the protein definitely is lessened.

Effect of Concentration of Milk and Storage Temperature on Protein Stability in Frozen Storage

Increasing concentration of the milk and increasing storage temperatures previously have been reported as decreasing the stability of the milk protein during frozen storage. The results obtained in this study are in accord with previous results and are tabulated in table 5. These are averages of six trials at various forewarming temperatures using paired samples.

Effect of Added Salts on Protein Stability in Frozen Storage

A large batch of concentrated skim milk was divided into six parts. Each of five parts received additions of one of the following salts, dissolved in the water of standardization: calcium acetate, sodium citrate, disodium phosphate, sodium hexametaphosphate, and sodium tetraphosphate. The amounts added were sufficient to produce a M/600 concentration of the respective salt on the basis of the volume of milk. This was repeated with concentrations of M/300 and M/150. The variously treated samples and a control then were frozen, stored at +5° F. to accelerate the action and examined at intervals as usual.

TABLE 5

The volume of flocculated protein in frozen and stored concentrated skim milk as influenced by concentration and storage temperature
(average of six trials)

Weeks in storage	At −5 to −15° F.		At +5° F.	
	Concentrated 3:1	Concentrated 4:1	Concentrated 3:1	Concentrated 4:1
Unfrozen	0.25	0.50	0.25	0.50
0	0.25	0.50	0.25	0.50
2	0.50	0.50	8.00	10.50
4	0.50	0.75	11.25	15.00
6	0.50	1.00	15.00	14.00
8	1.00	1.50	16.25	14.00
10	2.50	4.25		
12	4.25	5.75		
14	5.75	8.00	17.00	16.00
16	6.50	14.00		

Protein flocculation was not affected by calcium acetate, was slightly retarded by sodium citrate, but was considerably inhibited by the calcium sequestering salts (polyphosphates) which contributed a slightly objectionable astringent flavor at the highest concentration used. The results of this study with the M/150 concentrations are shown graphically in figure 4.

If, as has been suggested, protein flocculation in frozen storage is due to a "salting out" effect, it is difficult to visualize how relatively slight additions of salts of the type used would exercise any material influence on the stability of the milk protein because the water of the milk must be at or near the saturation point of several naturally-occurring salts. It appears, however, that the suppression of the calcium ion is an effective stabilizing factor even under these extreme conditions.

Effect of pH Adjustment on Protein Stability in Frozen Storage

Two trials were made in which the pH of the concentrated skim milk was increased by 0.4 and decreased about 0.4 by means of sodium hydroxide and lactic acid, respectively, added in the water of standardization. The results of the two trials were almost identical with respect to the effects on the development of the protein flocculate in frozen storage and on its ability to be redispersed with heat. The experience with one trial is presented in table 6.

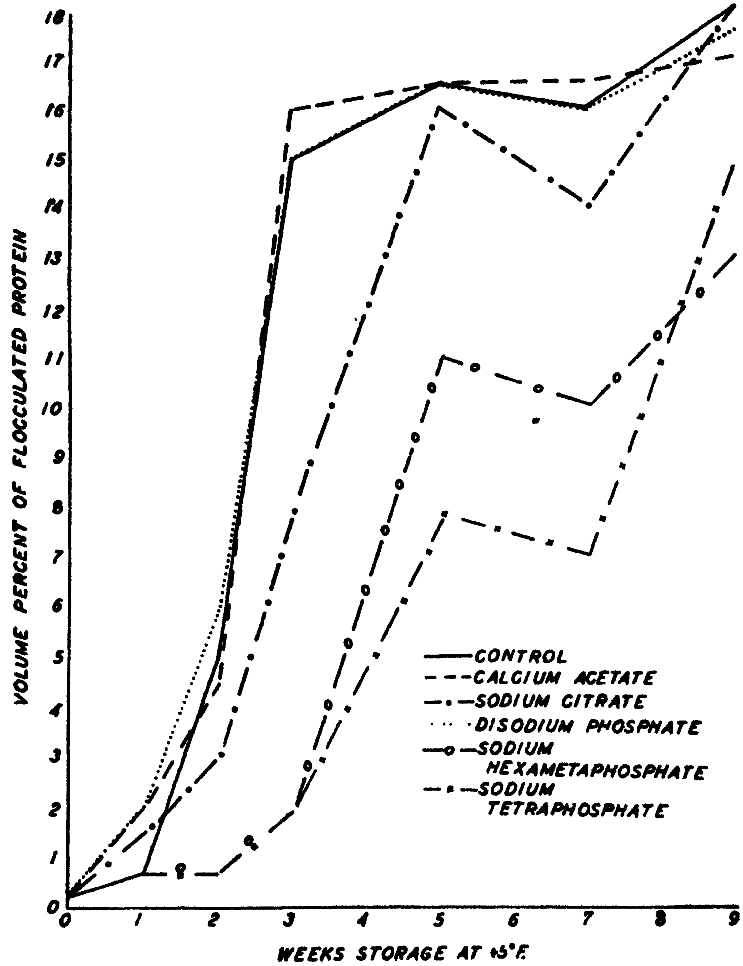

FIG. 4. The volume of flocculated protein in frozen and stored concentrated skim milk (3:1) as influenced by additions of various salts in M/150 concentrations.

Either increasing or decreasing the pH prior to the frozen storage of milk caused a slight but hardly significant acceleration of the rate of flocculation. Heat reversibility of the precipitation, however, very definitely was hindered by acidification and was favored slightly by alkalization. These results point to the importance of avoiding the use of high acid milk or milk which has undergone significant bacterial action when a product possessing qualities of long storage life is desired.

SUMMARY AND CONCLUSIONS

Freezing, in itself, produces no measurable flocculation of the protein of concentrated skim milk which is not immediately reversible on defrosting and reconstitution. Frozen storage, however, causes a gradual change in the protein properties whereby, with time, the reversibility is lessened and a floc can be separated and washed by centrifugation. In the early stages this floc is redispersible with time or immediately by heat or agitation. In extended frozen storage the precipitated protein eventually loses completely its reversible character.

TABLE 6

The volume of flocculated protein in frozen and stored concentrated skim milk (3:1) and its ability to be redispersed by heat as influenced by pH adjustments prior to freezing and storage at + 5° F.

Concentrated milk before freezing
Control—pH = 6.40
Alkalized—pH = 6.79
Acidified—pH = 6.02

Weeks in storage	Control—reconstituted pH = 6.62			Alkalized—reconstituted pH = 7.00			Acidified—reconstituted pH = 6.30		
	Cold	Heated—150° F.	Heated—200° F.	Cold	Heated—150° F.	Heated—200° F.	Cold	Heated—150° F.	Heated—200° F.
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Unfrozen	0.25			0.25			0.25	0.25	
0	0.25			0.25			0.25	0.25	
1	0.50	0.25	0.25	2.50	0.25	0.25	1.50	0.75	1.00
2	15.50	0.25	0.25	20.00	0.25	0.25	17.00	2.50	4.00
3	15.00	0.25	0.25	16.50	0.25	0.25	16.00	10.00	10.00
4	15.00	0.25	0.25	16.50	0.25	0.25	15.50	14.00	11.00
5	16.50	0.25	0.25	18.50	0.25	0.25	16.00	13.00	12.00
7	15.50	1.00	0.50	17.50	0.50	0.25	15.00	14.00	11.00
9	18.00	1.00	0.50	20.00	0.50	0.25	17.00	14.00	14.00
12	19.00	2.00	0.75	19.00	0.50	0.25	17.50	15.00	15.00

Calcium caseinate "salted-out" of milk exhibits identical properties and behavior, when stored at ordinary temperatures in the brine, to those of the flocculated protein separated from milk after frozen storage. Qualitative tests of the two precipitates also are identical. It is concluded, therefore, that the floc of frozen milk is calcium caseinate which has been thrown out of its normal colloidal dispersion by a "salting out" action of the high concentration of milk salts in the unfrozen water of the product.

Neither freezing concentrated skim milk nor holding it in the frozen state causes any change in titratable acidity, pH, or alcohol stability when determined after defrosting and reconstitution. Whatever alterations may exist in the frozen state, they are reversed quickly on defrosting.

As has been noted previously, flocculation of the casein is accelerated by concentration of the milk, higher storage temperatures, and longer periods of storage. It also is accelerated by the use of temperatures over 170° F. (76.6° C.) when applied as forewarming treatments in the process of concentrating the milk. Temperatures up to this point do not seem to cause any significant variations.

Calcium acetate and disodium phosphate in concentrations up to M/150 do not affect the rate of flocculation. Sodium citrate slightly decreases it, and the calcium-sequestering polyphosphates definitely retard it but introduce a detectable astringent flavor.

Neither raising nor lowering the pH of concentrated skim milk prior to frozen storage significantly alters the rate of casein flocculation. Both accelerate it very slightly. However, the reversibility of the coagulation with heat is decreased decidedly when the milk is acidified and is increased slightly when alkalized. This emphasizes the importance of avoiding bacterial action in milk to be frozen and stored commercially.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. I. TOCOPHEROL LEVELS IN THE COLOSTRUM AND IN THE EARLY MILK[1]

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The elucidation of the role of colostrum in the nutrition of the dairy calf suggested a study of the tocopherol concentration in this secretion. Such an investigation has received little attention. Mason (4) and Mason and Bryan (5) investigated this problem in rats by means of bioassays. They found that tissues of suckling rats from 24 to 48 hours of age were three to four times higher in vitamin E than were those of the newborn, indicating that colostrum of the rat was a rich source of tocopherols. Quaife (7), using a chemical procedure, found that early human milk had a higher tocopherol content than later milk.

In an investigation of the nutritional significance of vitamins A and E for the dairy cow, an opportunity was presented for studying the tocopherol content of the mammary secretions during the period of transition from colostrum to normal milk. The results of this phase of the investigation are reported herein.

EXPERIMENTAL

Grouping, feeding, and management of experimental animals. Dairy cows and heifers of four breeds, Holstein, Ayrshire, Jersey, and Guernsey, were grouped on the basis of the quantity of supplemental tocopherols added to the ration (table 1) and according to the management of the cows. Group I included all the animals that did not receive supplemental tocopherols[3] and were subjected to standard herd management. Since a survey of the data, as indicated in subsequent results, revealed no marked effect of vitamin A[4] supplementation on tocopherol concentrations in colostrum, the cows supplemented with vitamin A only were included in this group. Group II received vitamin A in addition to tocopherols; Group III received only tocopherol supplements. The cow in group IV received no supplemental vitamins and was milked throughout the gestation period. Details of the

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[3] Tocopherols in a soybean flour base were supplied by Distillation Products, Inc., Rochester, N. Y.

[4] Three forms of vitamin A concentrates were used: (a) vitamin A alcohol in a soybean flour base, (b) vitamin A alcohol in an oil medium, and (c) natural vitamin A ester in an oil medium. The first product was supplied by Distillation Products, Inc., Rochester, N. Y., and the latter two by the Borden Co., New York, N. Y.

quantities of vitamins given and the periods of supplementation are presented in table 1.

Vitamin A was included in this investigation, since the data presented herein were supplementary to a basic study of the effects of tocopherols on vitamin A and carotene in dairy cattle. All these supplements, except tocopherols in excess of 1 g., were administered *per os* in gelatin capsules; the larger amounts of tocopherol were combined with the concentrate mixture immediately before feeding. When both vitamins A and E were given, they were premixed and administered in the same capsule. Supplementation was discontinued at parturition. The rations were consumed readily by all animals except no. A–200, which refused some concentrate feed when the tocopherol supplement was introduced; however, the scheduled level of intake was resumed about 2 weeks before calving.

TABLE 1

Supplements given to pregnant cows daily

Group	Sub-group	No. of animals	Days previous to date of expected parturition	
			28–14	14–0
I	a	5	No supplement	No supplement
	b	6	500,000 I.U. vit. A alc.	1,000,000 I.U. vit. A alc.
	c	7	500,000 I.U. vit. A ester	1,000,000 I.U. vit. A ester
II	...	6	500.000 I.U. vit. A alc. and 0.5 g. α–γ-tocopherol	1,000,000 I.U. vit. A alc. and 1 g. α–γ-tocopherol
III	a	3	10 g. mixed tocopherol	10 g. mixed tocopherol
	b	1 (G–451)	0.5 g. α–γ-tocopherol	1 g. α–γ-tocopherol
	c	1 (G–467)	4 g. mixed tocopherol	4 g. mixed tocopherol
	d	1 (A–240)	5 g. α–γ-tocopherol	5 g. α–γ-tocopherol
IV	.....	1 (H–126)*	No supplement	No supplement

* Cow milked through gestation, without the dry-rest period.

The experimental subjects, except cow H–126, which was maintained on winter milking-herd rations, were fed a standard control diet consisting of a concentrate mixture (12.5 per cent crude protein), a good quality Atlas Sorgo silage, and high grade alfalfa hay. The concentrate feed was given at the rate of 1 lb. daily for 100 lb. of body weight, using the initial weight of the cow at the start of the trial as the basis for all subsequent feeding, which extended 7 days postpartum. Hay and silage were fed in the ratio of 1 : 2, the quantity being adjusted to the appetite of the individual cow. The animals were placed on the standard control ration approximately 6 weeks prepartal; the respective vitamin supplements were introduced into the diet 4 weeks before the date of expected parturition. Silage was fed in the morning, hay in the afternoon, and concentrates in equal portions at the morning and evening feeding periods. Whenever the weather permitted, the cows were released into an open lot, where water, salt, and bone meal were accessible.

Collection of colostrum and milk samples. It is recognized that as the milk is removed from the udder the fat content increases several-fold from the foremilk to the strippings. Since a similar increase has been observed for colostrum (6), the use of small samples collected directly from the udder may give an erroneous conception of the concentration of fat-soluble substances which apparently include the tocopherols. Therefore, special precautions were taken to obtain samples that were representative of the entire mammary secretions at various stages.

To prevent removal of colostrum by the newborn calf, the udder of the parturient cow was covered. The calf was segregated from its dam within 4 hours after birth and was not permitted to nurse at any time. A further measure to insure representative sampling was emptying the udder as completely as possible by standard milking procedures, including stripping. (This, contrary to popular belief, apparently did not predispose the dams to milk fever.) The mammary product, colostrum or milk, was mixed thoroughly before aliquots were taken for analysis. The first colostrum was obtained usually less than 2 hours after calving and always within 6 hours. Subsequent samples were taken at the regular morning and evening milking. The first four collections were analyzed individually, and the following collections, on the third, fourth, and eighth days, were analyzed as daily composites.

Analytical procedures. The method for determining the tocopherol concentration differed from that used by Quaife (7) only in the following respects: The samples for analysis ranged from 10 to 30 ml., depending upon the concentration of tocopherols. The removal of the Skellysolve B to obtain the residue containing the tocopherols was accomplished by evaporation under suction, using a water bath at 70° C. for heating. A Coleman spectrophotometer with a special cell holder that accommodated 7 × ½ inch absorption tubes was used in the assay. Readings were made at a wave length setting of 520 mμ. The instrument was calibrated using pure natural α-tocopherol.[5]

Since colostrum often is high in carotenoid pigments, care was taken to decolorize the extract completely by hydrogenation; sometimes it was necessary to use smaller aliquots to accomplish this. Occasionally fats tended to separate from the final hydrogenated extract. This precipitated material redissolved if the absorption tube containing the extract was placed in a water bath at 35° C. and shaken gently prior to the addition of the analytical reagents.

Milk fat content was determined by the standard Babcock procedure.

Method of expressing results. Not only was considerable variation found in the fat content of the mammary secretions from different cows during the colostric period but also from milking to milking in the same individual. To illustrate the possible effect of this latter factor upon the results when considering either a single or a few individuals, the tocopherol data for a typical group (I–c) and for an individual (G–468) which shows considerable deviation from the group average, are expressed in both γ/100 ml. of fluid and γ/g. of fat (fig. 1). The group averages (similar to those of the other experimental groups but not shown) indicate that the normal tendency is for tocopherol concentration to change from milking to milking in a definite manner. This decrease, as indicated in the subsequent discussion, has been found to follow the exponential law (fig. 2). The graph for cow G–468 (fig. 1) illustrates how the seemingly natural trend, as judged from the average, sometimes may be obscured unless tocopherol concentration is related to fat content. Consequently, tocopherol values are expressed herein as concentration per unit of fat.

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[5] Supplied by M. L. Quaife, Distillation Products, Inc., Rochester, N. Y.

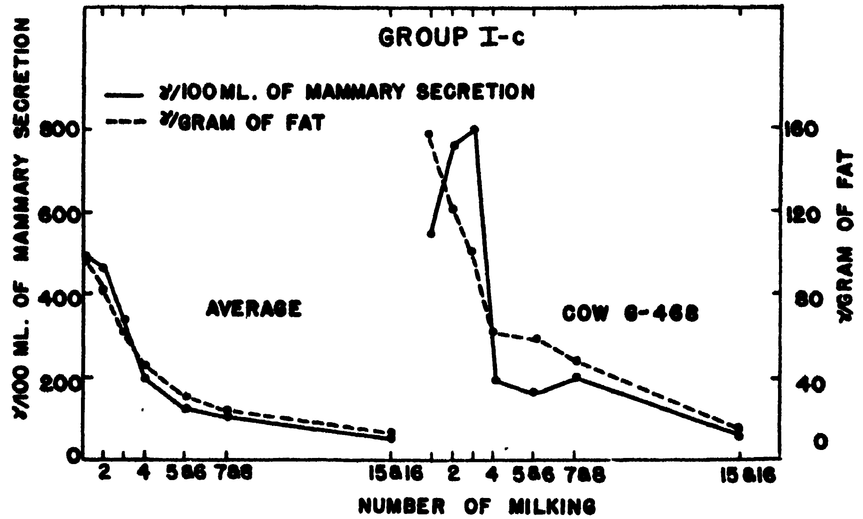

FIG. 1. Tocopherols in colostrum and in milk expressed as concentration per unit of fat and as concentration per unit volume.

RESULTS AND DISCUSSION

An examination of the results, shown in detail in table 2, reveals that there was considerable individual variation in the tocopherol content of the mammary secretions of cows within the same dietary group. The limited number of observations did not indicate any appreciable differences ascribable either to the breed of cow or to the number of previous lactations. However, additional data will be required to establish fully the influence of these aforementioned factors. While the first sample of colostrum usually had the highest tocopherol content per unit of fat, the maximum value for several cows was reached in the second milking.

The data of table 2 indicate that within group I, the average tocopherol content of the colostrum removed in the first milking was the highest for subgroup b and lowest for subgroup a, the average of the latter being almost the same as that for subgroup c. The average values for each of the three subsequent milkings were lowest for subgroup c; but as a whole, no striking differences are evident when values for the various samples from each of the three subgroups are compared. Apparently the addition of vitamin A to the basal diet had no definite effect on the level of tocopherols in the colostrum.

Tocopherol concentration in early colostrum was influenced by the levels of tocopherol intake (table 2 and fig. 2). Supplementation with 0.5–1 g. of tocopherols (group II) increased the concentration of this substance in colostrum 1.4 times the average level for cows not receiving this vitamin (group I). When 10 g. of tocopherols were given daily (group III–a), the concentration of these substances in first colostrum was 4.5 times that found for unsupplemented cows (group I). The ratios of the tocopherol levels in the first sample of colostrum of the foregoing groups of cows were essentially the same as those for blood serum tocopherol levels of the respective groups at the time of parturition (3).

There was a tendency for cows supplemented at the higher levels to maintain a greater concentration of tocopherols in the mammary secretions over a longer period of time than did those having a lower intake. Cow A–200 (table 2, group III–a), which refused to consume all the supplement in the early stages, was an exception to the general trend.

The low tocopherol values for H–126, which was milked throughout the gestation period, are in sharp contrast to those having the rest period. This suggests that normally within this period the cow accumulates reserves of tocopherols which rapidly are eliminated during the colostric period to provide for specific needs of the calf. However, the tocopherol requirements, which have not been established for the calf, may be less than the quantity normally obtained through ingestion of colostrum.

No data on the tocopherol content of silage have been found, but the grains and hay of the daily basal ration fed the cows probably contained from 0.5 to 1.4 g. of tocopherols, based upon the limited data of Cabell and Ellis (1). Presumably on a basal diet of lower tocopherol content the difference in tocopherol levels of colostrum from supplemented and unsupplemented animals would be greater than observed in this study.

In comparing the tocopherol concentration of the first mammary secretion with that of the eighth day, it was found that for cows receiving no supplemental tocopherols (group I), the average level of these substances was seven times higher in the first secretion than in the latter. Similarly,

TABLE 2

Tocopherol concentration in the fat of colostrum and of milk at different stages in the transformation period

Group	Subgroup*	Ration supplement	No. of lactation	No. of milking						
				1	2	3	4	5 & 6	7 & 8	15 & 16
				Tocopherols, γ/g. of fat						
I	a									
	H–175	None	6	94	77	71	57	50	25	24
	H–105		4	80	94	74	62	40	22	16
	A–230		3	107	117	70	..	53	29	17
	G–433		5	109	100	76	62	36	17	16
	G–432		5	101	98	76	63	26	46	26
	Subgroup mean			98	97	73	51	41	28	20
	b									
	H–115	Vitamin A alc.	2	52	46	34	25	16	12	11
	A–252		1	151	136	115	74	38	40	27
	A–243		2	111	85	84	65	41	25	10
	J–365		2	175	87	75	48	30	21	11
	G–465		1	148	139	65	54	28	14	.. ..
	G–452		2	93	82	56	40	30	15	16
	Subgroup mean			122	96	72	51	31	21	15
	c									
	H–132	Vitamin A ester	1	103	84	55	35	29	.. ..	.. ..
	H–128		2	91	78	48	42	21	22	12
	A–241		2	73	63	50	27	23	19	12
	J–378		1	64	79	66	57	42	24	18
	J–358		2	136	108	78	68	43	22	8
	G–468		1	157	122	102	63	59	48	14
	G–453		2	73	54	37	15	16	12	13
	Subgroup mean			100	84	62	44	33	25	13
Group mean				107	92	69	52	35	25	16

TABLE 2—(*Continued*)

Group	Subgroup*	Ration supplement	No. of lactation	No. of milking						
				1	2	3	4	5 & 6	7 & 8	15 & 16
				Tocopherols, γ/g. of fat						
II	H-135	Vitamin A alc. and 0.5–1 g. tocopherols	1	152	143	123	58	36	26	16
	H-136		1	150	114	90	65	44	26	16
	J-374		1	143	144	106	89	54	41	12
	J-380		1	143	140	124	75	81	47	16
	G-458		2	126	125	119	88	37	10	20
	G-459		2	184	122	107	81	43	32	21
Group mean				150	131	112	76	49	30	17
III	a									
	H-138	10 g. mixed tocopherols	1	412	296	234	194	135	87	43
	A-200		7	406	454	373	205	123	49	17
	J-356		2	648	705	520	358	287	158	58
	Subgroup mean			489	485	376	252	182	98	39
	b G-451	0.5–1 g. tocopherols	2	182	205	87	60	52	21	15
	c A-240	5 g. tocopherols	2	224	243	150	100	63	39	15
	d G-467	4 g. tocopherols	1	426	390	266	284	170	124	34
IV	H-126†	None	2	26	28	21	22	20	21	19

* The symbols H, A, J, and G refer to the breeds, Holstein, Ayrshire, Jersey, and Guernsey, respectively; the symbol is followed by the herd number of the cow.

† Cow milked through gestation; no dry-rest period.

for cows on the lower level of supplementation (group II), the initial value was nine times higher, and for cows receiving 10 g. of tocopherols (group III–a), 12 times higher.

Tocopherols in the colostric fat tended to decrease logarithmically during the first 4 days of the transition from colostrum to normal milk (fig. 2).

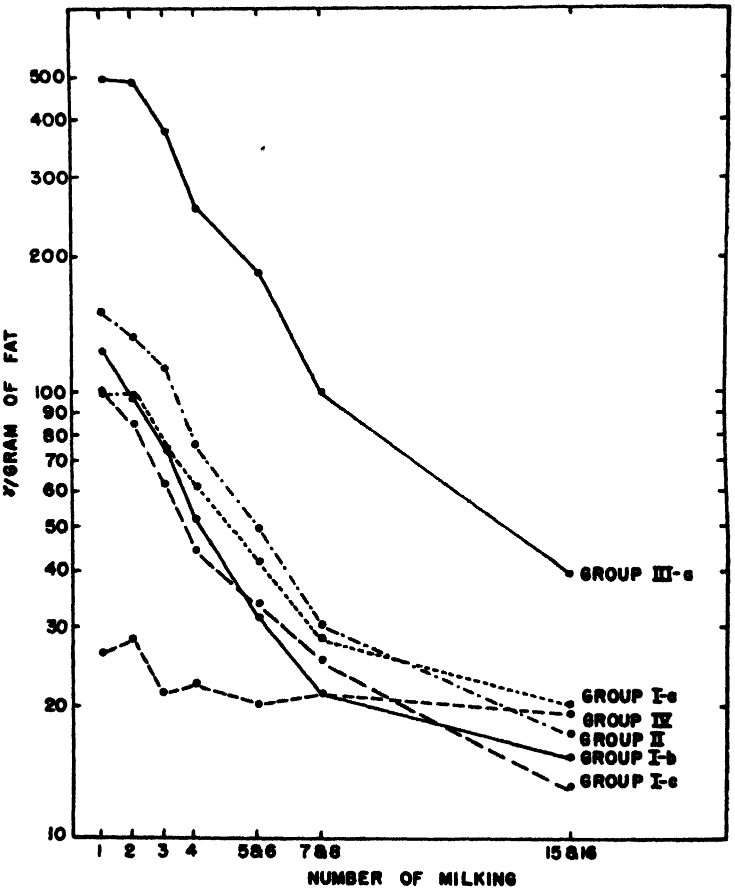

FIG. 2. Changes in concentration of tocopherols in the fat during the transition from colostrum to normal milk (concentration is plotted on a semi-log scale). The first milkings averaged approximately 3 hours postpartum. Group I–a received no vitamin supplements; group I–b received vitamin A alcohol; group I–c received vitamin A ester; group II received vitamin A and 0.5–1 g. of tocopherols; group III–a received 10 g. of tocopherols; group IV received no vitamin supplements and was milked throughout the gestation period.

Subgroup I–a and group III–a included several cows that secreted the highest concentration of tocopherols at the second milking; with these groups the logarithmic decrease commenced after the second milking. The rates of change of tocopherols in the colostric fat were similar for all groups, the change per gram of fat being between 0.18 and 0.26 γ/milking/γ. In the case of cow H–126 the change was rather erratic, but the general trend suggested a rate of only one-sixth to one-ninth of the foregoing rates. Group III–a, which received 10 g. of tocopherols daily, tended to maintain the initial rate of decline for a longer period than did the other groups. Except for group III–a, after about five to seven milkings a less rapid rate of decline in tocopherol concentration is observed (fig. 2). This condition probably resulted from two factors acting simultaneously: depletion of tocopherol reserves, and continued intake of tocopherols in the standard daily ration consumed during the postpartum period. If a cow were placed upon a tocopherol-free ration at parturition, probably the initial rate of change of tocopherols in the milk fat would be maintained for an extended period, and lower levels would be reached.

As previously indicated, the logarithmic trend seems to be most distinct for individual cows when tocopherols are expressed as concentration per unit of fat rather than as concentration per unit volume of mammary secretion. This suggests that the mammary output of tocopherols depends upon the quantity of fat secreted per unit of time as well as upon the other physiological processes involved in the mobilization and secretion of tocopherols. The indication of a relationship of fat and tocopherols in the mammary secretions is of further interest in light of the evidence, summarized by Hickman and Harris (2), leading to the conclusion that tocopherols, unlike vitamin A, are not associated primarily with fat in plant and animal organisms.

Table 3 shows the total average quantity of tocopherols secreted at the various stages of the colostric period by the cows in each experimental group. The total tocopherol output of the first eight milkings also is given. The range of values again emphasizes the wide individual variations. In general, the differences in the averages for cows of the various groups are similar to those already pointed out in table 2, except the average differences between groups I and II are smaller than when tocopherols are calculated as concentration per unit of fat.

It is of interest to consider the quantity of tocopherols a calf receives during the colostric period. If the calf nurses and, as usual, does not empty the mammary gland, it receives the secretion of lower fat content in a variable and indeterminable quantity. But if it is assumed that a 100-lb. calf consumes daily 10 lb. of a mixed aliquot of a complete milking from its dam, calves from dams receiving no tocopherol supplements (group I) would obtain from 22 to 86 mg. of this vitamin during the first 4 days. If the dams received tocopherols at the 0.5–1 g. level (group II), the calves would obtain 61 to 84 mg. of this vitamin, averaging 30 per cent more than the former group. On the same basis, calves fed colostrum from cows receiving 10 g. of tocopherols daily (group III–a) would consume about five times as

TABLE 3

*Total tocopherols secreted during the colostric period**

Group	Subgroup	Ration supplement		No. of milking						Total first eight milkings
				1	2	3	4	5 & 6	7 & 8	
				Tocopherols, mg./milking						
I	a	None	Av.	38	16	13	19	30	19	135
			Range	20–68	12–21	8–19	14–23	17–39	13–32	92–162
	b	Vit. A alc.	Av.	38	30	26	16	18	14	142
			Range	9–59	10–65	12–38	7–32	8–30	8–23	88–211
	c	Vit. A ester	Av.	39	20	18	11	17	14	119
			Range	8–68	6–32	9–25	4–16	12–23	9–33	79–160
II	–	Vit. A alc. and 0.5–1 g. tocopherols	Av.	47	22	30	24	26	18	167
			Range	4–99	3–52	13–60	12–35	17–38	8–30	89–262
III	a	10 g. mixed tocopherols	Av.	142	93	77	80	135	67	594
			Range	56–220	71–124	45–104	37–104	64–246	29–133	467–833
	b (G–451)	0.5–1 g. tocopherols		81	57	31	15	28	16	228
	c (G–467)	5 g. tocopherols		102	72	36	18	62	29	319
	d (A–240)	4 g. tocopherols		69	80	62	54	74	49	388
IV	(H–126†)	None		6	5	4	6	10	11	42

* First eight milkings.

† Cow milked through gestation; no dry-rest period.

much of these substances as would calves from the unsupplemented dams. The calf of cow H–126, which had no dry-rest period, would have received less than one-half as much tocopherol as a similar calf from a dam subjected to standard herd management, which included a rest period of at least 6 weeks.

Interestingly, by the fourth day cow H–126 secreted into 10 lb. of milk practically as large a quantity of tocopherols as the average of all experimental animals not supplemented with tocopherols (group I) and 60 per cent of the quantity secreted by cows receiving these substances at the 0.5–1 g. level (group II). In contrast, cows receiving 10 g. of mixed tocopherols (group III–a) supplied an average of four times more of this vitamin during the fourth day than did cow H–126.

At birth the tocopherol content of the blood serum of the calf was low but increased markedly upon ingestion of colostrum, the magnitude of the response varying with the tocopherol content of the colostric secretion (3). The response of the calf substantiates the direct measurements reported herein and is in accord with rat investigations (4, 5).

SUMMARY

Tocopherol levels were determined in the mammary secretions of dairy cattle during the period of transformation from colostrum to normal milk. The limited data revealed no differences that could be attributed either to the breed or to the number of previous lactations.

For cows receiving tocopherols at 0, 0.5–1 g., and 10 g. levels of supplementation during the latter stages of gestation, the concentrations of these substances in the first colostrum were in the ratio of 1, 1.4, and 4.5, respectively. The average levels of tocopherols in the first colostrum from the same three groups were, respectively, 7, 9, and 12 times higher than in the milk on the eighth day.

The change in tocopherol concentration in colostric fat followed a logarithmic trend that was similar for each of the experimental groups. Cows on the higher plane of supplementation tended to maintain the initial rate of change over a longer period of time than did those on a lower level of tocopherol intake.

For the cow that was milked through the gestation period, tocopherol levels of the first postpartum secretions were much lower and the rate of decline was less than for cows having the normal dry-rest period. However, values for this cow were practically as high by the end of the colostric period (4 days) as were those of cows not receiving supplements of tocopherols but allowed the dry-rest period.

Calculations were made showing the quantities of tocopherol that a calf would receive during the colostric period through consuming colostrum from cows on the various levels of supplementation.

The authors wish to acknowledge technical assistance of A. W. Neff, Grant Moody, and J. W. Mudge.

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PARTURIENT PARESIS. I. THE INCIDENCE OF PARTURIENT PARESIS AND CHANGES IN THE TOTAL BLOOD SERUM CALCIUM AT PARTURITION IN PREPARTUM MILKED COWS[1]

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Dryerre and Greig (2) postulated that the marked decline in blood serum calcium at parturition was the result of the sudden initiation of lactation. Turner (7) suggested that prepartum milking may reduce the incidence of parturient paresis or milk fever by a gradual initiation of lactation. In order to provide more conclusive information on this subject, an experiment was designed to ascertain the effects of prepartum milking on incidence of parturient paresis and upon the level of total blood serum calcium.

EXPERIMENTAL METHODS

This experiment was conducted on the Jersey herd of Biltmore Farms, Biltmore, North Carolina. At present the herd consists of approximately 600 milking cows grouped into ten functional units. A maternity barn is maintained for calving cows for three of the units. With the exception of first-calf heifers, practically all of the cows that calved at the maternity barn for 7 consecutive weeks (February 28 to April 17, 1947, inclusive) were prepartum milked. Eleven cows calving at outlying units during the same period also were included.

An effort was made to milk all cows 10 days prepartum based on the expected calving date and depending on when the cow was placed in maternity quarters. Usually prepartum milking began before the udder showed evidence of congestion. The cows were hand-milked twice daily. Milk production weights were taken for each prepartum milking. Calves were left with their dams from 1 to 3 days.

Blood samples were drawn daily from 3 to 5 days before the anticipated day of parturition, and usually 3 days subsequent thereto. Ordinarily samples were drawn between 8:00 and 9:00 a.m. In the event of parturient paresis, a blood sample was drawn immediately before treatment. Cows were not treated until definite comatose symptoms were evident. The blood serum samples were shipped in refrigerated cartons three times weekly to Madison, Wisconsin, for analysis. Total serum calcium was determined by the method of Clark and Collip (1).

A summary was made of the total number of calvings and the incidence

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TABLE 1

Changes in the total blood serum calcium at parturition in prepartum milked cows

Cow no.	No. of partu-rition	Days milked prepartum	Lb. milk produced day previ-ous to parturition	Total blood serum calcium (mg.%)						
				Days prepartum			Day of parturition	Days postpartum		
				3	2	1		1	2	3
16	3	11	24.7	10.3	9.7	9.7	8.9	8.2	9.4	10.0
38	2	14	25.1	9.5	9.3	9.6	9.4	9.3	10.1	10.5
47	2	3	8.5	*	*	*	6.6	6.6	8.9	10.1
50	2	6	10.5	*	9.0	9.0	8.6	8.3	10.1	10.2
56	2	6	12.5	10.7	10.6	9.8	9.3	8.5	9.4	10.6
112†	8	12	17.6	8.9	9.5	9.9	6.6–5.0‡	9.9	8.7	10.1
113	2	4	7.3	*	*	*	8.8	8.9	11.1	10.6
129†	6	12	8.0	11.1	10.7	*	3.9‡	3.7	8.8	9.7
130	2	8	20.2	10.8	10.3	9.0	8.7	9.0	10.6	10.2
132	10	8	16.0	7.3	6.5	9.0	10.0	7.8	9.6	*
151	2	9	23.5	10.4	10.4	9.4	9.2	9.8	10.1	10.2
163	6	10	17.0	9.4	10.9	12.5	8.7	9.2	9.5	9.9
168	8	5	11.7	9.8	8.7	9.5	7.7	7.6	8.7	9.3
203	2	10	6.0	10.4	8.9	9.9	9.0	8.6	8.3	10.7
205	2	14	§	10.2	10.8	9.9	9.4	10.2	10.2	10.7
225	2	5	1.3	9.9	10.2	9.7	*	7.7	9.2	9.1
236	2	11	14.2	*	10.7	12.2	9.7	8.6	9.1	*
249	2	5	0.9	10.5	11.2	10.5	9.8	9.3	10.1	10.0
267	5	4	0.4	*	10.4	10.9	10.7	10.2	9.2	7.7
284	6	6	1.4	10.2	10.4	10.7	8.3	5.8	9.2	10.1
321†	5	10	3.1	10.8	10.6	11.0	6.1–3.8‡	5.4–4.0‡	7.8	9.2
354	5	13	24.1	9.9	9.5	8.9	7.1	7.6	10.0	10.1
367	5	13	6.5	*	*	*	6.5	7.9	9.2	9.4
372†	5	11*	11.0	11.1	11.0	10.1	6.5–4.5‡	*	8.0	8.8
410	5	11	23.0	10.0	10.0	11.0	8.7	8.1	*	10.5

TABLE 1—(*Continued*)

Cow no.	No. of partu-rition	Days milked prepartum	Lb. milk produced day previous to parturition	Total blood serum calcium (mg.%)						
				Days prepartum			Day of parturition	Days postpartum		
				3	2	1		1	2	3
435	5	3	§	*	9.8	9.5	8.3	9.2	10.1	10.5
445	4	7	15.4	*	*	8.8	8.4	8.8	8.7	9.6
447	4	16	27.0	9.6	10.0	9.7	8.9	9.1	8.9	9.7
450	4	13	8.7	10.6	12.4	11.5	7.8	8.5	*	*
464†	5	8	5.2	*	*	9.4	6.1–4.0‡	6.1	5.7	*
512†	4	11	12.7	*	10.0	11.4	8.7–5.9‡	8.4–5.9‡	6.7	10.5
520	4	6	9.1	10.7	9.4	9.5	7.4	8.4	9.5	*
541	4	7	31.0	7.2	9.0	8.6	7.7	9.7	10.0	*
575†	6	9	1.1	10.9	*	10.1	6.9–3.9‡	5.9	*	8.5
577	3	2	6.2	11.8	10.3	9.7	9.1	10.2	11.7	10.4
580	4	2	§	*	10.0	9.5	8.6	9.6	10.3	10.6
583	3	14	32.2	9.8	9.6	9.4	8.0	8.5	10.4	*
588	3	6	15.5	11.8	10.2	9.3	9.8	8.9	9.4	10.3
590	4	3	§	9.2	10.2	10.2	9.6	9.0	10.5	11.1
595	3	15	23.4	*	8.9	9.5	9.4	8.6	10.3	10.4
597	3	2	0.3	*	*	*	8.6	7.2	9.7	9.6
M30†	4	9	0.5	11.1	11.4	9.2	5.5	5.1–4.9‡	8.0	*
M41	4	15	6.3	11.1	10.1	9.3	6.4	7.6	*	*
M82	8	8	11.5	10.9	10.7	10.4	7.8	6.5	8.9	*
M104†	6	7	16.2	*	*	*	*	*	*	*
R	?	6	2.0	11.0	11.6	11.3	8.7	10.0	*	*

* Either blood sample was not drawn, or the sample accidentally destroyed.
† Had parturient paresis.
‡ Blood sample taken before treatment for parturient paresis.
§ Milk not weighed.

of parturient paresis of cows calving at the maternity barn for the 7 weeks prior to and subsequent to the period when the cows were prepartum milked.

An analysis was made of the health records of cows calving at the maternity barn for the years 1943 through 1946, inclusive, excluding first-calf heifers. The herd was under the supervision of a veterinarian during the entire period, and complete records of all treatments were kept by the attendant at the maternity barn.

For the purposes of this study a "normal calving" will indicate there were no symptoms of parturient paresis.

RESULTS

The results of the study are presented in tables 1, 2, and 3. Forty-six cows were prepartum milked for 2 to 16 days. On the day previous to parturition, milk production ranged from 0.3 to 32.2 lb., with an average of 13.0 lb. Nine of the forty-six, or 19.6 per cent of the cows, had parturient paresis. Average milk production the day previous to parturition for those cows having parturient paresis was 8.4 lb., and for cows with normal parturitions, 13.4 lb.

TABLE 2

The effect of prepartum milking on the incidence of parturient paresis

	Period of:		
	Control	Prepartum milking	Control
	Jan. 10–Feb. 27	Feb. 28–Apr. 17	Apr. 18–June 5
Total parturitions	45	46	33
Parturient paresis	11	9	11
%	24.4	19.6	33.3

The incidences of parturient paresis during the 49-day periods before and following the period of prepartum milking are presented in table 2. Of the 45 cows that calved in the 49-day period just preceding that in which prepartum milking was done, 11 or 24.4 per cent had parturient paresis. Thirty-three cows calved in the first 49 days immediately following the period of prepartum milking, of which 11 or 33.3 per cent had parturient paresis. The difference in the incidence of parturient paresis during the prepartum milking period and control periods is not statistically significant by the chi-square test of independence and association. The incidence of parturient paresis in the same herd for the 4 previous years was 19.3 per cent (table 3).

Cow 112 appeared lethargic the morning of the day of parturition, and her condition grew progressively worse toward evening, at which time she was staggering. The attending veterinarian diagnosed her condition as parturient paresis. Subsequent analysis showed the total serum calcium level to be 5 mg. per cent. A posterior pituitary extract was given, and delivery of the calf was manually aided.

Cow M104 was housed at one of the outlying units and blood samples were not obtained. This cow was unable to arise the day after parturition, and her condition was diagnosed by the veterinarian in charge as parturient paresis. She responded to calcium gluconate therapy.

Thirty-five of the forty-four cows on which total serum calcium was determined showed a definite drop in the calcium level the day of or day after parturition. Three other cows, 47, 113 and 367, had relatively low total serum calcium levels on the day of parturition, but previous blood samples were not obtained, so no comparison could be made. Cows 132 and 541 had relatively low total serum calcium levels before parturition.

TABLE 3

Incidence of parturient paresis of cows calving at maternity barn of Biltmore Farms (1943 through 1946, inclusive, excluding first-calf heifers)

Months	Total no. of parturitions	Parturient paresis	Parturient paresis
			(%)
Jan.	111	26	23.4
Feb.	130	38	29.2
March	123	13	10.6
April	105	27	25.7
May	105	27	25.7
June	97	15	15.5
July	111	23	20.7
Aug.	108	22	20.4
Sept.	63	8	12.7
Oct.	72	7	9.7
Nov.	81	10	12.3
Dec.	77	12	15.6
Total	1183	228	19.3
May–Sept.	484	95	19.6
Oct.–April	699	133	19.0

DISCUSSION

A comparison of tables 1, 2, and 3 shows that prepartum milking as performed in this study did not reduce the incidence of milk fever. In some cows, milk secretion was not stimulated by prepartum milking, even though milking began several days before parturition. Although average milk production was not as high in cows having parturient paresis, milk flow was well established in five of these cows—112, 129, 372, 512, and M104. With cow 112, parturient paresis occurred before parturition. Preparturient paresis is not uncommon, as statistics compiled by Jensen (5) showed that parturient paresis occurred before parturition in 4.24 per cent of 1,107 cases.

There were no marked differences between the summer and winter months on the incidence of parturient paresis in this herd (table 3). Generally, these cows are turned to pasture the first 2 weeks of March. Parturient paresis occurred just as frequently in the months from May through September as from October through April. This observation is in agreement with Hibbs *et al.* (4), but at variance with Metzger and Morrison (6) and Henderson (3).

SUMMARY

Forty-six cows were prepartum milked from 2 to 16 days. Nine of these cows had parturient paresis.

Prepartum milking did not significantly reduce the incidence of parturient paresis.

It would seem from the results of this study that factors in addition to the initiation of milk secretion are involved in causing parturient paresis.

The incidence of parturient paresis is not seasonal in this herd.

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THE EFFECT OF FEEDING CERTAIN SYNTHETIC VITAMINS OF THE B COMPLEX ON THEIR CONTENT IN MILK AND URINE

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It is generally believed that the B vitamins are not essential in the diet of mature ruminants for normal growth, lactation, and reproduction. Wegner *et al.* (18) have demonstrated the ability of the bovine to synthesize a significant amount of thiamin, riboflavin, pyridoxine, biotin, nicotinic acid, and pantothenic acid when fed a ration very low in these factors. McElroy and Goss (9, 10) indicate that thiamin is not a dietary essential for ruminants, but that the cow and the sheep synthesize this vitamin in the rumen and reticulum. The work of these investigators indicated the synthesis of thiamin, riboflavin, pyridoxine, and pantothenic acid in the ruminant. These vitamins were reported as being formed either in the body tissue or by microorganisms in the rumen. Nicotinic acid also was reported as being synthesized in the ruminant by Winegar *et al.* (20). Bechdel *et al.* (1) found the rumen microflora to be 90 per cent flavobacterium (their own designation). This organism was fed to rats after having been grown in a B complex-free media and found to be highly potent in the vitamin B complex. This probably explains why cattle can produce normal offspring and milk on a ration that is deficient in the vitamin B complex for rats.

Williams *et al.* (19) determined eight vitamins of the B complex in milk from different species. They reported the vitamin levels in the different milks generally to increase progressively from human beings and the mare to the mouse. This increase in the content of these vitamins in milk of the different species is analogous with the rate at which these species reach maturity. This would seem to indicate that the B complex is a factor in growth rates. Pearson and Darnell (12) showed that colostrum from either the cow or the ewe was considerably higher in thiamin and riboflavin than was the normal milk of the respective species. Since nature provides an abundant supply of these two vitamins, possibly they are essential for early development and growth of young mammalia. There also are seasonal variations in thiamin and riboflavin, as well as ascorbic acid, as shown by Holmes *et al.* (4). The change from a dry roughage to silage during the months of December and January caused a sharp increase (36 per cent within 2 days) in riboflavin content in Jersey and Holstein milk as shown by Theophilus and Stamberg (17). Considerable work has been done on feeding diets low in B vitamin, synthesis by the ruminant, and the effect of ration on B vitamin content in milk. A survey of the literature reveals no work concerning

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the feeding of these vitamins in the synthetic form and their effect on the contents in the milk. Since milk constitutes an important item in our diet, experimental work was undertaken to study this problem in dairy animals.

EXPERIMENTAL

Work was started with milking cows. Goats were used later to reduce the amount of synthetic vitamins fed and to permit the use of a metabolism cage for the collection of urine. A purebred Jersey cow and a purebred Holstein cow were selected from the college dairy herd. Since according to Pearson and Darnell (12) the amount of certain B complex vitamins is higher in early lactation, cows and goats were selected that were far enough along in lactation to assure normal milk. Previous lactations of these animals were checked and found to be normal.

The goats used in this experiment were reported by their previous owners to be purebreds—one a Saanen and the other a Toggenburg. They were stall-fed without access to pasture throughout the experiment. The concentrate ration for each species was the ration used for the milking herd. A fair quality alfalfa hay was used, along with sorghum silage; however, the cows had access to pasture and as the experiment ran through the spring flush season, some changes in the intake levels of some of these vitamins may have occurred [Holmes *et al.* (4)].

Preliminary studies were completed on April 11, 1946, and the cows were placed on rations that had been supplemented with 500 mg. each of synthetic thiamin, riboflavin, nicotinic acid, and pantothenic acid. All four of these vitamins were fed concurrently to the cows as well as the goats. Daily collection of samples began with the first milking on April 12, 1946. Collections were made at this level for a period of 6 consecutive days; then the supplement was increased to 2 g. daily and collections were taken for 10 consecutive days at this level. A further study was conducted at a 16-g. level. During this period one cow was fed 16 g. of each of the vitamins for a period of 3 days. Samples of milk were taken on the second and third days and assays run on the samples.

Goat no. 1, a Toggenburg, was placed in the metabolism cage for a period of 2 days on May 28, 1946, and a preliminary study was made while the goat was on the basal ration. In this case samples of milk, urine, and blood were taken and vitamin determinations were run on each of these products with the exception that thiamin was not determined in blood. On June 7, goat no. 1 was placed in the metabolism cage for a period of 10 days and given the basal ration plus 2 g. of each of the vitamins for the first 5 days, no supplement being given during the last 5 days. This gave a study period of 12 days during which time daily collections of milk and urine were made. The blood samples were taken on the second, seventh, and twelfth days during the study period. Goat no. 2, a Saanen, was given treatment identical to that of goat no. 1. Further study on each goat was made at a low level of 250 mg., which is approximately equivalent to the 2-g. level for the cows, based on body weights of the two species. During this period of 4 days the goats were fed 250 mg. of each of the vitamins, and composite samples were made from daily collections of milk and urine. The blood samples were taken on the third day.

The cows and goats were milked twice daily. Samples were taken from a.m. and p.m. milkings and a composite made for that day. The urine was collected from the goats by means of the metabolism cage and blood samples taken from the jugular vein. To prevent photochemical destruction of riboflavin (15), all samples of milk and urine were collected in brown bottles and stored in the dark at a temperature of 0 to 4° C. All laboratory work with this particular vitamin was carried out under subdued light.

Thiamin was determined by the fluorometric method of Hodson (3). Riboflavin was determined by the microbiological method of Snell and Strong (14), with a slight modification devised by Isbell (6). Nicotinic acid was determined by the microbiological method of Krehl *et al.* (8), with a slight modification devised by Isbell (6). Pantothenic acid was determined by the microbiological method of Strong *et al.* (16), as modified by Neal and Strong (11).

RESULTS

The data on the vitamin content of the milk from the cows are summarized in table 1. The average thiamin content of the milk of the Jersey and Holstein while on the basal ration was 44 μg. and 34 μg. per 100 ml. for the respective animals. These values compare favorably with the values ranging from 41 to 53 μg. per 100 ml. for milk from a mixed herd of dairy cows (5). The thiamin values during the period of supplementation with 500 or 2,000 mg. daily of thiamin were not significantly different from the values on

TABLE 1

The effect of the ingestion of thiamin, riboflavin, nicotinic acid, and pantothenic acid on the levels in the milk

(Values in μg. per 100 ml.)*

Vitamin supplement	Jersey			Holstein		
	Values on basal ration	Levels of vitamin ingested		Values on basal ration	Levels of vitamin ingested	
		500 mg.	2000 mg.		500 mg.	2000 mg.
Thiamin	44	38	42	34	36	39
Riboflavin	178	198	167	98	103	88
Nicotinic acid	104	123	119	82	90	89
Pantothenic acid	341	329	399	275	268	309

* Values on basal ration average for 2 days, values during periods of vitamin supplementation average for 6 days.

the basal ration, indicating that the feeding of synthetic thiamin is not effective in increasing the concentration in the milk. One cow was force-fed 16 g. of thiamin without any significant increase in the concentration of this vitamin in the milk. The milk of the goats fed 250 mg. of thiamin did not show any increase in thiamin level over the period during which the basal ration was fed. When 2 g. of thiamin were fed daily to goats, there was a significant increase in the level in the milk and a very marked increase in the amount excreted in the urine (fig. 1).

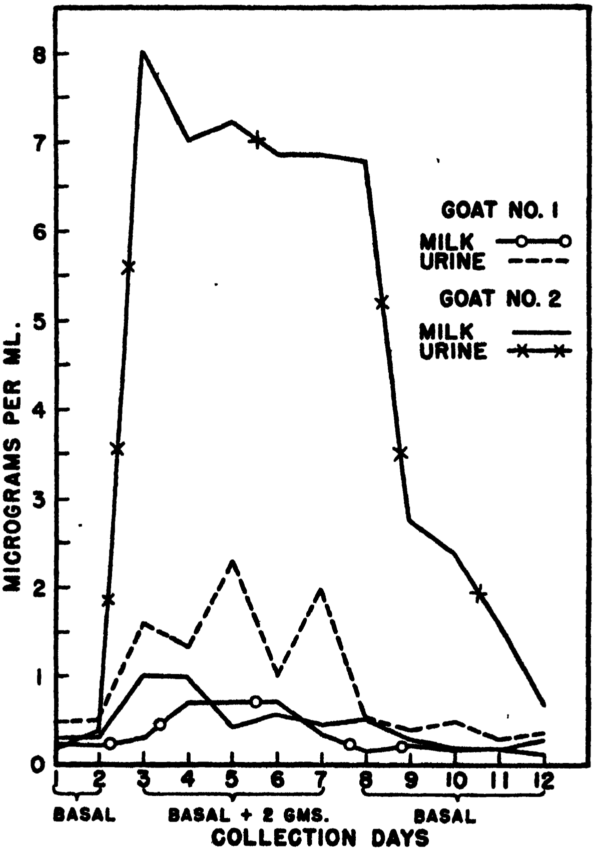

FIG. 1. The effect of the daily ingestion of 2 g. of thiamin by goats on the levels of thiamin in the milk and urine.

The feeding of synthetic riboflavin, nicotinic acid, and pantothenic acid to cows at levels of either 500 or 2,000 mg. daily for periods of 6 days did not increase significantly the concentration of these vitamins in the milk. The feeding of a massive dose of 16 g. of each of these three vitamins was without appreciable effect in increasing the concentration of these vitamins in the milk. The milk of the cow fed 16 g. of riboflavin had a distinct greenish-yellow color characteristic of aqueous solutions of riboflavin. Milk from the cow and goats fed the higher levels of riboflavin gave a much higher value for riboflavin when determined fluorometrically than when measured microbiologically. An account of this phase of the work is reported in a separate paper (13).

The daily feeding of 250 mg. of riboflavin, nicotinic acid, and pantothenic acid did not result in a significant increase in the concentration of any of the vitamins in the milk. The feeding of 2 g. daily of each of these vitamins to goats resulted in a significant increase in the concentration of each in the milk and a very marked increase in the amount in the urine (figs. 2, 3, and 4).

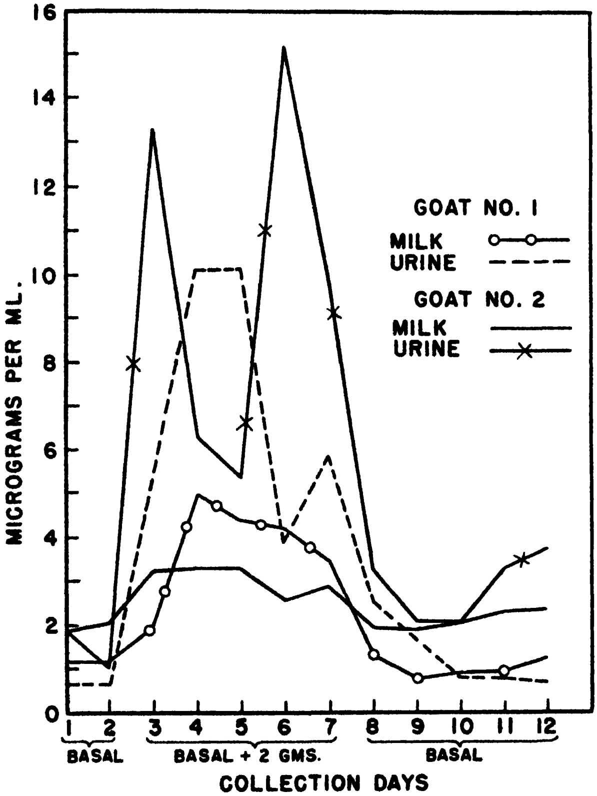

FIG. 2. The effect of the daily ingestion of 2 g. of riboflavin by goats on the levels of riboflavin in the milk and urine.

DISCUSSION

The possible destruction of the water-soluble vitamins, as in the case of ascorbic acid, must be kept in mind [Knight *et al.* (7)]. There appeared to be no significant increase in content in cow's milk of any of the four vitamins studied at the level fed in these studies. An analysis of variance of the data was conducted to determine breed differences in milk levels relative to the four vitamins studied. In each case there was found to be a significant breed difference on a unit basis. The average values observed were: thiamin, 44 μg. per 100 ml. in Jersey milk as compared to 34 μg. per 100 ml. in Holstein milk; riboflavin, 198 μg. per 100 ml. in Jersey milk and 98 μg. per ml. in Holstein milk; nicotinic acid, 104 μg. per 100 ml. in Jersey milk and 82 μg. per ml. in Holstein milk; and pantothenic acid, 341 μg. per 100 ml. in Jersey milk and 275 μg. per 100 ml. in Holstein milk. These values were for normal milk while the cows were on the basal ration. As shown, the Holstein ran consistently lower than the Jersey on a milliliter basis; however, on total daily milk production, the Holstein cow had a much greater output than the Jersey. This agrees with similar work with riboflavin by Theophilus and Stamberg (17).

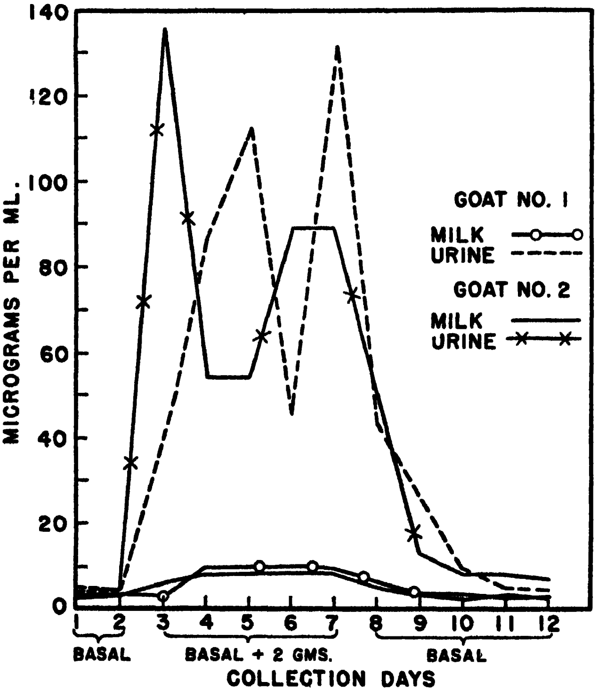

FIG. 3. The effect of the daily ingestion of 2 g. of nicotinic acid by goats on the levels of nicotinic acid in the milk and urine.

In comparing the vitamin content in milk of the two species, the following observations were made on a unit basis and by using averages of the two breeds of each species. For thiamin the goats were found to have 29 μg. per 100 ml. compared to 36 μg. per 100 ml. for the cows. This figure is contradictory to a report by Gamble *et al.* (2), in which they state that Toggenburg and Saanen milk contained 1 I.U. of thiamin in 3 to 4 ml. as compared to 1 I.U. of thiamin in 5 to 7 ml. of Holstein and Jersey milk. This is in agreement with work by Williams *et al.* (19). Riboflavin content in the goat milk was 157 μg. per 100 ml. as compared to 138 μg. per 100 ml. for the cows, which is in agreement with work by Gamble *et al.* (2) based on rat feeding assays. The values for the goats are slightly higher than the value given by Williams *et al.* (19), which was 110 μg. per 100 ml. The nicotinic acid content for goat milk was found to be almost three times as high as for cow's milk, the values being 267 μg. per 100 ml. for goats and 93 for cows. Pearson and Darnell (12) reported the average nicotinic acid content for cow's milk as 91 μg. per 100 ml., and Williams *et al.* (19) reported goat's milk as having 250 μg. per 100 ml. This work agrees with those reports and certainly indicates a very distinct species difference in levels of nicotinic acid in the milk. The pantothenic acid content was found to be 322 μg. per 100 ml. in goat's milk compared to 306 μg. per 100 ml. in the cow's milk. This work indicates no species difference in pantothenic acid content of milk and agrees with work of Pearson and Darnell (12) in which they report 367 μg. per 100 ml. in cow's milk. Williams *et al.* (19) report the value for goat's milk as 240 μg. per 100 ml., which is only slightly lower than values reported in the present study.

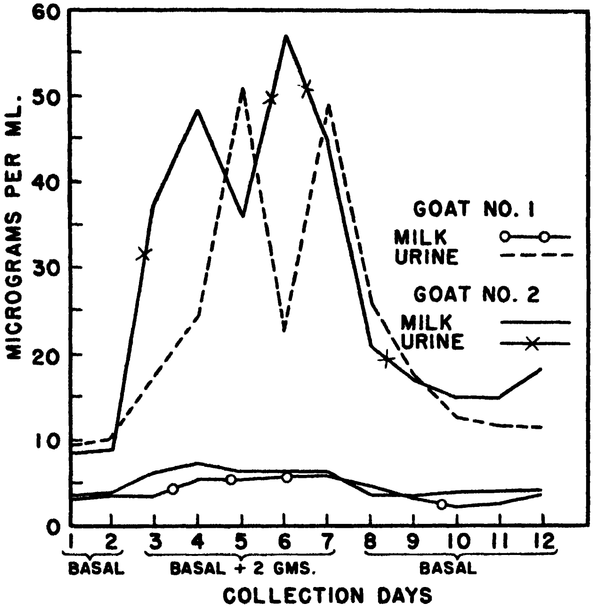

FIG. 4. The effect of the daily ingestion of 2 g. of pantothenic acid by goats on the levels of pantothenic acid in the milk and urine.

Assays were made on the blood for riboflavin, nicotinic acid, and pantothenic acid by the microbiological procedures. Blood samples were collected from the goats before, during, and after the feeding of the vitamins at the 2-g. level. The blood values for riboflavin ranged from 21 to 36 μg. per 100 ml. There was no consistent difference in the riboflavin content of the blood of the goats during the period they were on the basal ration and the period they were receiving riboflavin. The ingestion of the nicotinic acid increased the level on the basal ration from 560 to 624 μg. to a maximum of 948 and 1,034 μg. per 100 ml. of blood for the respective goats. The pantothenic acid values on the basal ration were 78 and 65 μg. per 100 ml. of blood. During the period of pantothenic acid feeding, the maximum values for pantothenic acid were 140 and 184 μg. per 100 ml. of blood.

SUMMARY

1. Synthetic thiamin, riboflavin, nicotinic acid, and pantothenic acid fed at a 2-g. level daily to dairy cows resulted in no significant increase in the levels in the milk.
2. There appeared to be a distinct breed difference in the cows in amount of these B vitamins in the milk on a unit basis, the Jersey being higher than the Holstein in micrograms per milliliter, while the Holstein was higher in total amount secreted daily, based on total milk production.
3. In the case of the goats a significant increase of each of the vitamins occurred after they had been fed at the 2-g. level; partial destruction was indicated, since the per cent recovered in the milk and urine was 10 per cent or less of the amount ingested.

Acknowledgment is made to Merck & Co. for the synthetic vitamins used in these investigations.

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STUDIES ON MILK PROTEINS. I. CONDITIONING OF MILK PROTEINS WITH PANCREATIC ENZYMES

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When milk is treated with minute amounts of active pancreatic enzymes and pasteurized immediately, its proteins undergo modifications. This change is evidenced by the fact that hard, strong clots no longer can be formed through the use of acids or coagulating enzymes such as pepsin or rennin. Conquest *et al.* (1) have described this action in connection with milk of reduced curd tension. Casein primarily is affected according to the work of Turner (3), who has obtained a greater solubility in 70 per cent ethyl alcohol in this protein separated from pancreatic enzyme-treated milk than that found in a normal control.

The laboratories of Armour and Company have been interested in studying caseins precipitated from acidified, pancreatic enzyme-treated milks and have noted a change somewhat different from the above. These caseins appear to be affected by heat in a manner opposite to the untreated control. For example, temperatures in excess of 150° F. will cause the enzyme-treated casein to melt and flow, whereas untreated caseins become stringy, then hard, and finally glassy. Previous investigations reported by Conquest *et al.* (1), Doan and Flora (2), and Turner (3) have disclosed small chemical differences between pancreatic enzyme-treated milk and the untreated milk. Since these chemical changes seem diminutive when compared to the physical ones noted in the following experiments, other factors may be involved. In this connection one could predict molecular dissociation wherein a limited number of chemical bonds are ruptured. For purposes of studying this phase, the principles of osmosis have been utilized. Such technique, properly controlled, should reveal whether appreciable molecular dissociation or cleavage had transpired.

EXPERIMENTAL PROCEDURE

A cellophane tube listed as Sargent S–25275, 28 mm., was used. Sacs were formed from it by cutting 8-inch lengths and tying one end with a string. One-hole rubber stoppers were held in the tops of the cellophane sacs by glass bands cut from large tubes and slipped up over the sacs to give strong joints. Calibrated glass tubing of 7 mm. size was inserted in the stoppers for containing the liquid increase and for providing a means for its measurement.

The milk to be tested first was warmed to 25° C., and 70-ml. portions were measured into the cellophane sacs. The sacs were immersed at equal

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depths in running distilled water at 25° C. Readings of liquid column heights were made hourly. Determinations always were run parallel in order better to eliminate discrepancies due to variations in barometric pressure. Experimental portions were removed from a large lot of raw milk so that uniformity might prevail. A commercial preparation of pancreatic enzymes used for producing soft curd milk, and sold under the trade name of "Enzylac," was the enzyme source. Whenever used, it was added at the recommended rate of 1 : 10,000 or 0.01 per cent to the cold, raw milk. Pasteurization was started immediately and a temperature of 145° F. was maintained for 30 minutes according to standard procedure.

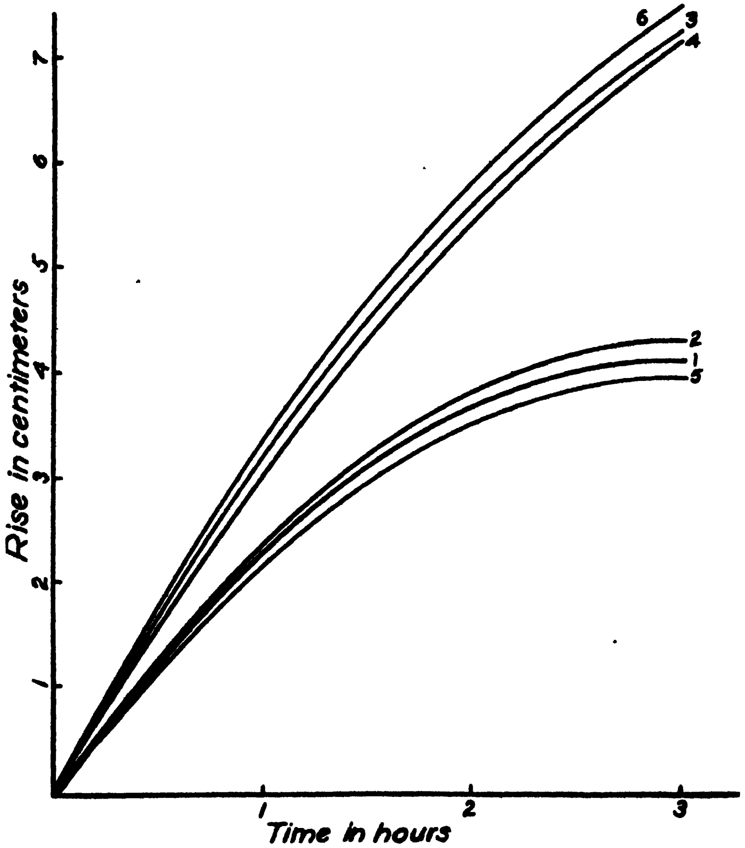

FIG. 1. Osmotic rates obtained on experimental milks at 25° C. 1. "Enzylac" enzyme added to the cold milk and then pasteurized. 2. Inactivated Enzylac enzyme added to the cold milk and then pasteurized. 3. Enzylac enzyme added to the cold milk, then pasteurized, when Enzylac enzyme again was added. 4. Milk first pasteurized and then Enzylac enzyme added. 5. Inactivated Enzylac enzyme added. 6. Enzylac enzyme added to milk; no subsequent treatment.

All digestive enzymes of animal origin are inactivated by heating in the range of pasteurization, so this step terminated further action of the Enzylac on milk proteins. Experimental milks employed in this study were made from one lot of raw milk.

The curves (fig. 1) represent typical osmotic rises, obtained on these milks, plotted against time, thereby showing osmotic rate.

RESULTS

Curve 1 shows Enzylac-treated milk with a curd tension of 25 to give the same rate of osmosis as milk which was pasteurized and had a curd tension of 60 (curve 2). Raw milk (curve 5) essentially is in a similar range with the other two samples.

Curves 3, 4, and 6 demonstrate the osmotic rate observed when digestion by active pancreatic enzymes is in progress. Obviously the increased rate of these milks goes far beyond the other samples.

From these osmotic studies it is apparent that milk treated with pancreatic enzymes and pasteurized is comparable to either pasteurized or raw milk with respect to numbers of particles contained in equal volumes. Thus, chemical differences between enzyme-treated milk proteins and controls would seem to be due to small disintegrations at the molecular surface rather than deep cleavages.

SUMMARY AND CONCLUSIONS

Apparently the pancreatic enzyme treatment recommended for making a milk of low curd tension induces a weakening of the internal structure of casein. In this condition the molecules are rendered susceptible to dissociation by heat. For descriptive purposes, this unstabilizing action is referred to as "conditioning".

Physical measurements rather than chemical methods seem to have greater possibilities for measuring this effect.

Work on both physical and chemical methods for determining degree of "conditioning" or change in molecular structure is being continued.

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STUDIES ON MILK PROTEINS. II. COLORIMETRIC DETERMINATION OF THE PARTIAL HYDROLYSIS OF THE PROTEINS IN MILK

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The need to detect and measure smaller degrees of protein hydrolysis than can be detected by available methods occurs frequently enough in the laboratory to warrant research on methods.

One problem under study involves the treatment of liquid milk with small amounts of pancreatin for the reduction of curd tension. The degree of breakdown of the protein structure cannot be determined by conventional methods, such as the Sorensen formol titration. Turner (4) reported a greater alcohol-soluble fraction from trypsin-treated milk. However, this method does not give results uniform enough to be translated into the degree of protein hydrolysis. Some methods require special equipment and techniques, whereas others use purified substrates that make them less adaptable to a complex mixture containing protein such as milk.

The method of Anson (1), using a denatured hemoglobin solution as a substrate to measure proteolytic activity of enzymes, was found to be the most adaptable for measuring the hydrolysis of milk protein. The enzymes liberate enough of the amino acids tyrosine and tryptophane from the hemoglobin so that when the phenol reagent of Folin and Ciocalteau (2) is added to a sample of the protein-free filtrate, a blue color is formed which can be measured colorimetrically.

EXPERIMENTAL PROCEDURES

1. *Phenol reagent.* To the reagent prepared according to Folin and Ciocalteau (2), two volumes of water are added. This is the same reagent that Kay and Graham use in their phosphatase test (3).

2. *Trichloroacetic acid.* A 0.72 N solution is prepared from reagent grade trichloroacetic acid.

3. *Sodium carbonate–sodium tetraphosphate solution.* Seventy-five grams of anhydrous sodium carbonate and 10 g. of sodium tetraphosphate (Quadrafos) are dissolved in water and diluted to 500 ml.

4. *Tyrosine standard solution.* Eastman tyrosine recrystallized and dried is used. A nitrogen determination should be run to determine the purity of the product. One hundred milligrams of the tyrosine are dissolved in water and enough formaldehyde is added to prevent bacterial growth; the solution then is diluted to 500 ml.

Methods. When milk is substituted for the denatured hemoglobin solu-

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tion, several changes have to be made in the reagents. The method of Anson (1) calls for a 0.3 N trichloroacetic acid solution to precipitate the protein, hemoglobin. The concentration must be increased to 0.72 N in order to remove the milk proteins.

In preparing the protein-free filtrate for addition of the phenol reagent, it is advisable to use a 15 per cent sodium carbonate solution containing enough polyphosphate to prevent the precipitation of the calcium salts of milk during the addition of the carbonate.

Since milk and enzyme preparations normally contain some color producing products, as shown by this test, blanks must be run on them in order to correct for this color formation.

A 5-ml. quantity of milk is pipetted into a clean dry test tube; 1 ml. of distilled water is added, followed by 10 ml. of 0.72 N trichloroacetic acid while agitating the test tube to mix the milk. The tube then is stoppered, shaken vigorously, and allowed to stand for 10 minutes before filtering the contents through an S&S no. 595 filter paper. Five milliliters of filtrate are added to a 50-ml. Erlenmeyer flask and 10 ml. of the sodium carbonate reagent added and mixed thoroughly before 3 ml. of phenol reagent are added. The sample is shaken continuously while adding the phenol reagent. About 5 minutes are allowed for the blue color to reach a maximum before any readings are made.

The blue color is measured in a Coleman spectrophotometer, using 650 mμ wave-length light. The color also can be measured satisfactorily by using a photometer with appropriate glass filters that transmit light in the 650 mμ wave-length range. A previously prepared standard tyrosine curve showing the per cent light transmission for various tyrosine concentrations then is used to convert the sample reading into its tyrosine equivalent.

Milk protein hydrolysis is demonstrated very readily by the addition of various amounts of pancreatic enzymes to the milk and allowing the enzyme to react for short periods. Five milliliters of milk are pipetted into the test tubes and the required amount of water added to make up the difference between the volume of the enzyme solution added and 1 ml. After bringing these test-tube samples to the desired temperature in a water bath, the required amount of enzyme solution is added. At the end of a 10-minute period the enzyme action is stopped by adding 10 ml. of trichloroacetic acid. A blank on the enzyme solution is prepared by adding the required amount of this solution to the substrate after the trichloroacetic acid has been added to the milk.

The standard tyrosine curve was prepared by adding various amounts of tyrosine to a series of test tubes, and making up to a total volume of 6 ml., followed by the trichloroacetic acid. The remainder of the procedure was the same as that used for a milk sample except that filtering was not necessary.

The concentration of tyrosine in the final 5-ml. aliquot used for producing the color may be up to 0.15 mg. without producing too much color to be measured accurately. By plotting the per cent light transmission against the tyrosine concentration (in the final 5-ml. aliquot) on semi-log graph paper, a straight line was produced.

RESULTS

The results obtained for producing the standard tyrosine curve are given in figure 1.

Some typical results of the action of a sample of Armour's Pancreatin U.S.P. upon milk are given in figure 2.

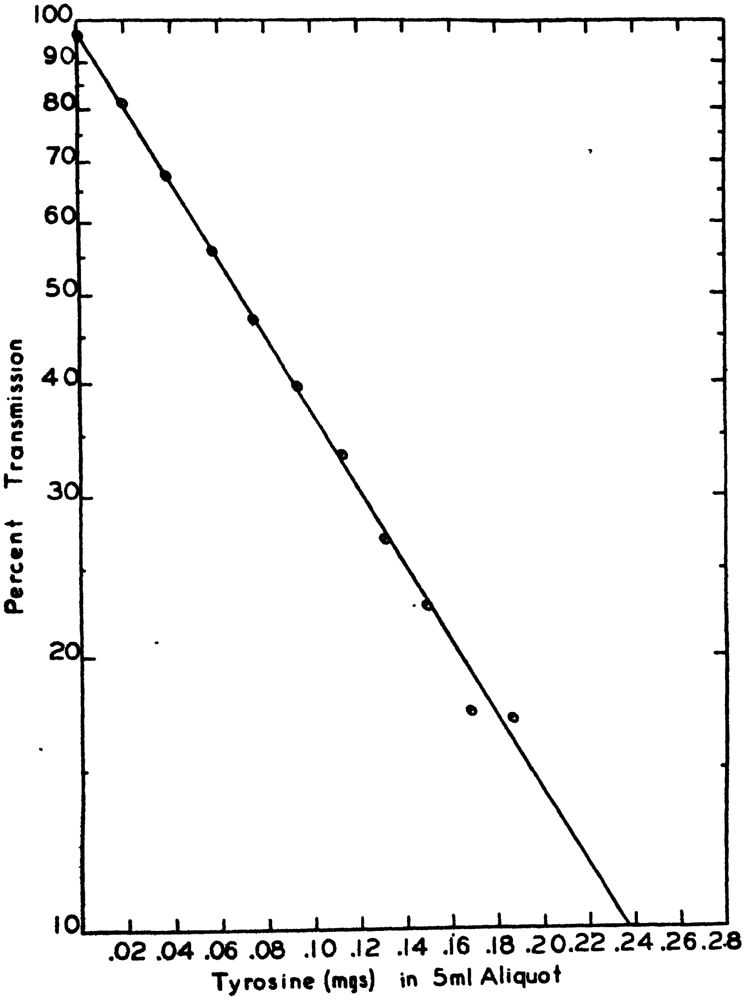

FIG. 1. Spectrophotometer measurement of color produced from tyrosine and phenol reagent at 650 mμ.

In calculating the results of this test, the tyrosine content is based upon its concentration in the final 5-ml. volume used for the production of the blue color. In making a determination, a sample of milk is treated as described, and the percentage of light transmitted is found. Locating this figure on the curve in figure 1 will give the tyrosine equivalent in milligrams in a 5-ml. aliquot sample.

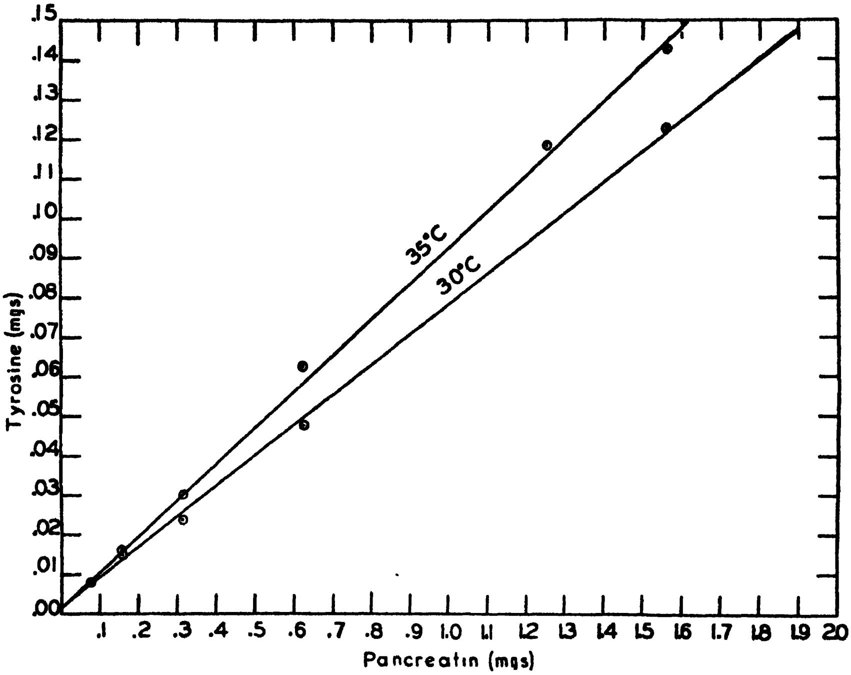

FIG. 2. Milk protein hydrolysis with pancreatic enzyme in 10 minutes.

CONCLUSION

A rapid colorimetric method can be used to measure minute amounts of milk protein hydrolysis. It is a very sensitive and a practical method for showing protein hydrolysis.

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STUDIES ON MILK PROTEINS. III. THE MODIFICATION OF MILK BY PANCREATIC ENZYMES

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The modification of milk by the use of pancreatic enzymes to give improved digestibility characteristics first was described by Conquest *et al.* (6) in 1938. Since that time, reports of clinical investigations (1, 2, 3, 4, 11), as well as results by various *in vitro* techniques (7, 10, 13, 14), have established the enzyme treatment as a suitable means of milk modification.

The active enzyme employed in the process is proteolytic and, if allowed to react for a sufficient period, will bring about pronounced protein hydrolysis. However, in the usual process, the enzymes are inactivated by heat after a relatively short time and the desired degree of modification is attained without altering the appearance or flavor of the milk or causing any substantial protein hydrolysis. The techniques common to protein study, such as the Kjeldahl analysis for total protein (6, 14), the determination of free amino acids by the Van Slyke method (13) or the formal titration (6), as well as studies of osmotic pressure (9) and conductivity (15), have shown no conclusive changes which would be associated with protein hydrolysis. Turner (13) did report, however, that the enzyme treatment caused an increase in the amount of protein soluble in 70 per cent alcohol. The lack of sufficiently precise techniques for studying protein degradation, as well as the fact that too little is known of the initial phases of enzyme activity, has made it difficult to offer a satisfactory explanation for the results obtained by enzymatic treatment.

A modified test for tyrosine, devised by Hull (8), has proved to be extremely sensitive for determining the breakdown of milk proteins. It seemed probable that this method could be applied to advantage in studying the enzyme treatment of milk and that it might make possible a more definite explanation of what had occurred. In the present experiment Hull's method was used as a means of determining protein degradation, with measurements of curd tension and protein stability included as criteria of the degree of treatment with pancreatic enzymes.

EXPERIMENTAL

Samples of enzyme-treated milk were prepared in the laboratory by adding appropriate amounts of a freshly prepared aqueous suspension of Enzylac Powder[1] to cold raw milk, heating the milk to 142° F. in 10 to 15

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[1] Pancreatic enzyme extract distributed by American Seal-Kap Corporation, Long Island City, N. Y.

minutes, holding at 142–145° F. for 30 minutes, and cooling in cold running water. Enzyme concentrations of 20, 40, 60, 80, and 100 p.p.m. were used in each series of samples; raw and pasteurized controls also were included.

Curd tension tests were performed in accordance with the method recommended by the American Dairy Science Association Committee on Curd Tension (5), using an American Curdometer.

The technique used for determining protein stability was that reported previously by the author (12), comprising briefly the addition of small increments of N/10 hydrochloric acid to 10-ml. portions of the sample in a series of test tubes, heating the tubes to boiling in a water bath, and then examining to determine the smallest amount of acid required to cause coagulation. The "Stability number" reported is equivalent to 100 times the ml. of N/10 acid used to produce coagulation.

Tyrosine tests were carried out as described by Hull (8). Color development was measured photoelectrically, using a Lumetron Model 402EF colorimeter with Corning Glass Filters no. 3962 and 2403 in the primary position. This filter combination has maximum transmission at a wave length of approximately 700 mμ, which is satisfactory for the purpose. Values for tyrosine were interpreted from a standard curve prepared from known tyrosine dilutions, using the same procedure as employed in the regular test. Amounts of tyrosine are expressed as "Tyrosine units", a unit being 1 mg. of tyrosine per liter of sample or its equivalent. The possibility of some other amino acids reacting in the test was considered, and a brief investigation indicated that the method was sensitive to tyrosine and tryptophane but not to cystine or phenylalanine. Considering tyrosine recovery in the test to be 100 per cent, tryptophane recovery was found to be approximately 88 per cent. Bearing in mind the relative amounts of these two amino acids in milk protein, it is believed that about 80 per cent of the blue color development in the test is due to tyrosine while 20 per cent is due to tryptophane. These figures are based upon empirical values and are cited in explanation of the use of tyrosine units as a means of expressing results.

RESULTS

Five series of samples were prepared. The average results of curd tension, protein stability, and tyrosine determinations are shown in table 1.

Statistical analysis revealed that, while both relationships were significant, tyrosine values were related more closely to protein stability than to curd tension. The following regression equations were obtained, with the correlation coefficients (r) as shown:

$$Y = 36.3 - 0.25x \qquad r = -0.936$$

$$Y' = 127 - 1.25x \qquad r = -0.963$$

in which Y = curd tension

Y' = protein stability number

x = tyrosine units

TABLE 1

Effect of added enzyme upon curd tension, protein stability, and tyrosine content of milk

(Average results—five series of samples)

Treatment	Curd tension	Protein stability no.	Tyrosine units
Raw milk	28.0	76	35.4
Pasteurized milk	27.0	82	36.2
Enzyme—20 p.p.m.	24.6	68	47.2
Enzyme—40 p.p.m.	22.4	56	57.2
Enzyme—60 p.p.m.	19.2	46	65.6
Enzyme—80 p.p.m.	18.2	36	73.4
Enzyme—100 p.p.m.	16.4	24	80.6

Av. time of heating to pasteurizing temperature was 13 minutes.

DISCUSSION

In view of the natural low curd tension of the raw milk which was available for experimentation, a feature not uncommon in the Chicago area, it would be unwise to attribute too much significance to curd tension relationships which were developed. The author feels that wider discrepancies might be found if milks of greater curd tension were examined, although the samples which were prepared were comparable with milks generally encountered in this market area. The value of curd tension as an index of the degree of enzymatic modification is lessened considerably in milk of this type, since the upper limit of 30 g. generally accepted in the industry for soft curd milk has little relationship to the treatment under such circumstances. Therefore, as an arbitrary index, it was felt that milk which had been processed in such a manner as to reduce the curd tension at least a third, or to about 20 g., could be considered as having been treated satisfactorily.

The effect of the enzyme action on the protein stability, on the other hand, offered a much more reliable index of treatment. Unpublished data by the author, including several years of practical experience with the commercial preparation of enzyme milk, have shown that satisfactory enzyme modification yields milk with a protein stability number in the range from about 20 to 45. Up to this time, the stability test has been the simplest and most convenient means of assaying treatment, although no claim has been made for it as an index of digestibility and it has served chiefly as a method of process control.

On the basis just described for deciding what constitutes satisfactory enzyme treatment, the data show that a curd tension of 20 g. corresponds to a tyrosine value of 65 units, whereas a protein stability number of 45 is equivalent to 65.6 tyrosine units. The correlation between the amount of tyrosine and these properties which have been used in the past as indices of enzymatic treatment suggests that the tyrosine value also might serve as a measure of the degree of modification. In fact, the tyrosine value might well be a superior means of measurement, since it must be the direct result of protein degradation and, hence, a true measure of protein modification rather than an indirect or arbitrary index. Therefore, a tentative limit of at least 65 tyrosine units might be considered as a suitable minimum level for proper enzyme modification.

The average tyrosine value found in untreated milk, both raw and pasteurized, was approximately 36 units. Although large numbers of determinations have not been made, it has been unusual to find normal untreated milk with fewer than 30 tyrosine units or more than 42. In any case, satisfactory treatment of milk by the enzyme process has resulted invariably in the production of at least 30 additional units of free tyrosine in the sample. This, then, affords a basis for an estimate of the amount of enzymatic protein hydrolysis. By calculation from empirical values, the liberation of 30 additional tyrosine units represents about 1 per cent of the theoretically possible total of all tyrosine and tryptophane in the milk which might be freed in a complete hydrolysis. Therefore, it is not surprising that less sensitive methods of analysis have failed to show any significant changes as a result of mild enzyme activity.

The work of Keil and Roundy (9) and others (6, 13, 14, 15) would indicate that mild enzymatic action brings about no gross changes in the milk protein, from which it may be concluded that the protein molecule has not been altered materially. However, there is no doubt that this relatively mild treatment does affect other properties of the milk, such as curd characteristics, which generally are not considered as appropriate measures of hydrolysis, if related at all. The zone within which enzymatic modification is accomplished falls within the very earliest stages of protein breakdown.

The results of this investigation indicate that a significant increase in tyrosine, if not other amino acids as well, can be shown by a suitably sensitive technique. This may seem incompatible with prior results if it is believed that hydrolysis proceeds more or less simultaneously throughout the protein mass. However, the general concept is that proteolytic enzyme activity is a surface phenomenon, with the action not penetrating deeply into the molecule. Since the protein molecule is large and complex, the proportion of it engaged in a surface reaction at any one time would be small; it is quite likely that pronounced hydrolysis of the surface layer could occur without bringing about changes great enough to be detected upon subjecting the entire mass to analysis. Under this assumption the remaining portion of the molecule still would be in essentially its original condition, considering the composition of the mass, although the surface characteristics probably would have been altered. These surface changes would be expected to involve the degree of hydration as well as the electrical charge of the particles, properties which are of primary importance with respect to the stability and coagulating characteristics of the protein.

The theory is advanced that the effects of enzymatic modification of milk are due principally to the action of the enzymes upon the surface of the protein. This can account for the lack of gross changes in the protein as well as the evidences of slight hydrolysis. Furthermore, through changes in surface effects, the modifications in curd characteristics which have been observed can be explained.

SUMMARY AND CONCLUSIONS

A sensitive test for tyrosine has been applied to a study of milk modified by the enzyme process, with results indicating that the free tyrosine content of the milk increases with the degree of enzymatic treatment. The relationship seems to be sufficiently satisfactory for the tyrosine test to be given consideration as a new measure of enzymatic modification of milk and a tentative level of 65 tyrosine units is suggested as a suitable minimum limit.

Relationships of the free tyrosine content of milk to the curd tension and the protein stability were determined statistically and found to be significant. The degree of correlation was highest for the tyrosine-stability relationship.

According to the amount of tyrosine found in properly treated samples, it is estimated that normal enzyme modification of milk accounts for hydrolysis amounting to approximately 1 per cent of the theoretically possible total.

A theory is offered suggesting that changes brought about on the surface of the protein molecules by the action of proteolytic enzymes are responsible for the modified characteristics of enzyme-treated milk.

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THE ISOLATION AND PRELIMINARY STUDY OF SOME PHYSIOLOGICAL CHARACTERISTICS OF THE PREDOMINATING FLORA FROM THE RUMEN OF CATTLE AND SHEEP[1]

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The importance of microorganisms in ruminant digestion generally is accepted, but little is known about the physiological activities of the predominating flora. This fact is emphasized in Hastings' recent review (3). Some studies have been carried out to determine the role played by rumen bacteria in urea utilization (7, 9, 13, 15), and in cellulose digestion (5, 8, 10, 14).

Studies were initiated in 1945 to develop techniques for the culturing of the predominating bacteria in order that their physiological activities could be investigated. The successful procedures include: (a) a direct slide counting technique using an indirect stain, to serve as a guide for the success or failure of the cultural work; (b) a method for obtaining a sample suitable for culture from a fistulated animal; (c) an anaerobic procedure for growing the bacteria in the numbers indicated by the direct count; (d) methods for studying urea utilization; and (e) methods for cellulose breakdown.

EXPERIMENTAL RESULTS

Technique for slide counts. The direct counting technique found most successful involved the use of nigrosine, which colored the background but left the bacteria unstained. Rumen contents were diluted with 1 per cent glucose solution to 1:1,000 or 1:10,000, depending upon the expected bacterial content of the sample, and shaken for 5 minutes at a speed of 240 oscillations per minute. The vigorous shaking was very essential in order to free the bacteria from the food particles and break up clumps of bacteria. After shaking, 0.01 ml. of the sample was transferred by a standard loop to a thoroughly cleaned slide upon which an area of 2 × 2 cm. had been marked off. A similar loopful of one-half saturated methyl alcohol solution of nigrosine was mixed thoroughly with the sample, and spread evenly over the 4 $cm.^2$ area. The slide was placed on a very hot electric plate so that drying occurred within 2–3 seconds. The slide now was ready to count, the bacteria appearing white against a black background.

Counts were made from several fields on three to five different slides;

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so that at least 400 cells were enumerated; the calculations were made according to Knaysi and Ford (6).

This technique was not intended to be extremely accurate but was employed in this study as a guide for the success or failure of the cultural results. The method used has the advantage of being rapid, and the counting is easy. It gives a permanent record of the organisms in the sample and overcomes difficulties encountered by other workers (7, 12). However, it has weaknesses. For example, both living and dead bacteria are counted, as they appear the same under the microscope. An attempt to overcome this difficulty by using a "vital" stain, following the procedure of Knaysi and Ford (6), did not appear satisfactory, as the bacteria died rapidly when exposed to air and some aeration could not be avoided in this technique.

Obtaining samples for cultural studies. The ideal subject for cultural work was found to be an animal with a fistula, since rumen contents could be obtained without undue exposure to air. Liquid samples from the rumen were taken by inserting a pipette into the bottom of the rumen, drawing up some of the liquid, and transferring it immediately into a stoppered flask nearly full of sterile 1 per cent glucose solution. The receiving flask was prepared by first autoclaving the glucose solution in a large flask and then transferring the solution to a sterile 1-l. flask which was filled nearly to capacity with 1,000 ml. of the glucose solution. After steaming for 1 hour, the flask was tightly stoppered and cooled rapidly in cold water. The cooled flask was taken to the experimental animal. The stopper was removed only long enough to receive the sample.

Solid samples of rumen contents were removed with a long-handled metal cup on which a tight-fitting rubber stopper was placed immediately after filling. One gram of this material was quickly weighed into a tared, sterile petri dish and transferred rapidly into a liter flask containing glucose solution.

Liquid samples taken by stomach tube and catheter from non-fistulated animals were studied by a similar procedure.

Procedure for culturing predominating flora. Success in culturing depended upon the continued maintenance of anaerobic conditions from the time of taking the sample until growth had occurred. From the rumen samples obtained, dilutions were made to 10^{-12}. Inoculations were made by pipetting the diluted sample into broth which previously had been placed for 10 minutes in a boiling water bath and then cooled rapidly to 45° C., immediately preceding inoculation. The tubes were sealed with vaspar directly after inoculation to maintain anaerobic conditions. Usually only the dilutions of 10^{-9} to 10^{-12} were inoculated, but occasionally 10^{-8} was included and 10^{-12} omitted. Speed is very essential in the whole procedure.

Several types of media were used in these experiments. Two media gave good results. A regular broth or agar had the following composition: 1 per cent each of tryptone, peptone, beef extract, glucose, and yeast extract; 2 per cent fresh skimmed milk (added separately after removing medium from boiling water bath); and 0.5 per cent dipotassium phosphate. If agar were included, 1.5 per cent was used. The pH was 6.0.

A meat infusion broth plus sugar, a modification of the broth of Smith and Sherman (11), contained the following nutrients per liter: 1 g. glucose, 10 g. tryptose, 500 ml. meat infusion, 25 ml. tomato juice, and 5 g. dipotassium phosphate. The pH was 6.5–6.6. Both media usually had cellulose added in the form of acid-treated cotton, prepared as suggested by Hungate (4).

In almost every case in the high dilutions, the smear showed only one morphological type of organism. To obtain pure cultures for testing the physiological characteristics, an agar shake was made from the broth, and isolated colonies were picked.

Incubation of the cultures was at 38° C. and daily observations were made. Growth manifested itself, either by a general turbidity throughout the tube or by a change in the appearance of added cellulose, which was characterized by clumping or a tendency of the cellulose to cling together in strings. In either case a smear showing organisms confirmed the presence of growth. Ordinarily growth would appear in dilution 10^{-9} in 48 hours, but in two or three cases it was necessary to wait 60 days for growth to appear in the higher dilutions. Subcultures in agar shakes showed that the organisms isolated from the top dilutions were anaerobic, since no growth took place nearer than 0.25 to 0.50 inch below the surface.

Although the predominating cultures isolated usually compared with the morphology of the predominating organisms as seen on the original slides from the rumen contents, in some instances cocci which seldom were isolated were present in large numbers. This would indicate that the cultural technique should be improved, probably in respect to maintenance of anaerobic conditions, because the cocci apparently would survive less aeration than some of the rods.

Table 1 summarizes the data showing the average bacterial slide counts and the highest dilution in which growth was observed from samples obtained by various methods from animals on a normal diet. The slide count figures reveal no essential differences between sheep and cattle in the bacterial population of their rumen contents. The solid samples from the fistulated animals of both species showed about the same average number of bacteria per gram, and the samples obtained from slaughtered sheep by forcibly squeezing the liquid out of the solid were similar to the figures for solid samples. Probably these latter samples more nearly represented a solid sample than a liquid one, due to detachment of bacteria which normally would adhere to the solid material. In both species the number of bacteria was somewhat lower in the liquid than in the solid samples.

TABLE 1

Comparison of average bacterial slide and cultural counts from samples of rumen contents obtained by various methods from animals on a normal diet

Sample	Type	No. of samples	Av. slide count	Av. slide count of samples in highest dilution showing growth			
			(billions per g. or ml.)	*(below 10^{-9})*	*(10^{-9})*	*(10^{-10})*	*(10^{-11})*
Cattle with fistula	Liquid	10	76 ± 10.3*	None	29 (1)†	64 ± 8.7 (5)	100 ± 15.5 (4)
	Solid	16	92 ± 8.3	None	29 (1)	76.5 ± 10.7 (4)	110 ± 7.9 (11)
Sheep with fistula	Liquid	4	62 ± 9.3	53 (1)	47 (1)	60 (1)	89 (1)
	Solid	4	93 ± 19.5	135 (1)	80 (1)	80.5 ± 32.5 (2)	None
Sheep by stomach tube	Liquid	16	27 ± 2.23	27 ± 7.2 (3)	27 ± 7.8 (11)	30.5 ± 3.5 (2)	None
Sheep slaughtered	Liquid expressed from solid	9	95 ± 5.7				

* Standard deviation.

† Numbers in parentheses indicate number of observations.

Athough the individual counts of the liquid and solid samples show considerable overlapping when both types of samples were obtained from the same animal at the same time, the bacterial count of the solid always was higher than that of the liquid sample except in one case, where they were equal. This would indicate that the liquid really did have a somewhat lower bacterial population than the solid.

It also can be seen from table 1 that the method of obtaining the sample was important. The liquid samples obtained by stomach tube gave a lower average slide count than the liquid samples obtained from fistulated sheep by pipette, which would indicate that these samples taken by stomach tube were not representative even of the liquid fraction of the rumen contents. Differences in animals and diets also may have influenced the results.

Data also are presented (table 1) which show that there is good agreement between the slide and cultural "counts" on samples obtained from the fistulated bull. In 24 out of 26 cases the samples grew in the dilutions expected from the slide count. This would indicate that success had been achieved in culturing the predominating organisms. However, such good agreement was not obtained with the fistulated sheep since in only three cases out of eight did the slide and cultural results agree. These results were better than those obtained with samples taken from the sheep by stomach tube, where only 2 out of 16 samples showed growth in the dilution indicated by the slide count.

As stated above, the maintenance of anaerobic conditions was essential for good cultural results, since most of the rumen organisms are anaerobic and the close agreement between the slide and cultural results obtained with the fistulated bull reflect the good cultural conditions available when working with this animal. The poorer agreement between the slide and cultural counts with samples from the fistulated sheep probably is due to the less favorable conditions prevailing in sampling the sheep. The aeration attendant upon taking a stomach tube sample would account for the poor results obtained in culturing this material. Aspirated samples appear poor because they did not give representative samples of the rumen contents and they permitted aeration of the material.

The slide and cultural counts found in this study are higher than any figures reported in the literature. The highest slide count reported was about 13 billion organisms per gram of fresh contents (7), while the average obtained in this study from the solid material from a fistulated animal approximated 100 billion organisms per gram. The highest cultural growth was reported by Hungate (5), who obtained growth of cellulose-digesting bacteria from rumen contents diluted one billion times when cultured in inorganic medium. In the present study bacteria, including many cellulose digesters, have been grown from a 1 : 100 billion dilution of rumen contents. The figure of 100 billion bacteria per gram of fresh rumen contents agrees well with the calculated figure of 100 billion bacteria as cited by Köhler (7). Further evidence in support of the probable accuracy of the counts reported here is the satisfactory agreement between the slide and cultural counts.

Test for urea utilization and cellulose digestion. To test for urea utilization the organisms isolated from dilutions 10^{-10} and 10^{-11} were inoculated into an inorganic salt broth (4), modified to contain 1 per cent glucose, 0.04 per cent urea (in place of ammonium sulfate), 20 µg. of each of the following vitamins—thiamine, riboflavin, pyridoxin, pantothenic acid, niacin, and paraaminobenzoic acid; and 0.05 µg. of biotin. The mixture of vitamins was autoclaved separately and added aseptically to each tube at the time of inoculation. The pH of the broth after all additions was 6.5. In order to produce and maintain anaerobic conditions, the tubes were treated and sealed as previously described. If the cultures survived three transfers in this broth, it was assumed that they were able to utilize urea.

In the early part of this work, in order to determine whether urea actually had disappeared in those cultures in which growth had occurred, determinations of total nitrogen and of urea were run on several broths which had been inoculated with low dilutions (10^{-3} and 10^{-4}) of rumen contents from lambs fed 40 per cent of their protein equivalent as urea. An uninoculated blank tube also was tested.

The determination of total nitrogen was carried out by the usual colorimetric procedure, digesting 1 ml. of the medium with acid digestion mixture and Nesslerizing. The urea was determined by the urease method on 1 ml. of the medium. This also was Nesslerized. All analyses were run in duplicate.

Some of the cultures isolated from 10^{-10} and 10^{-11} dilutions of rumen contents were tested for cellulose digestion by a modification of the technique of Fuller and Norman (2). The cultures were inoculated into the meat infusion broth prepared as previously described, except that only 0.01 per cent of glucose was included. The cultures were incubated from 2 to 4 weeks in a desiccator which had been evacuated and filled to one-half capacity with carbon dioxide repeated three times. All cultures were run in duplicate and the results of the two tests were averaged.

When incubation had been finished, the supernatant fluid was decanted through a tared Gooch crucible, filtered by suction, and the pH tested. The residual cotton in the test tube was treated with 10 ml. of 0.1 per cent sodium carbonate for 5 minutes to remove the bacterial gum which some cultures formed in the presence of glucose. Several washings were carried out with small portions of distilled water, followed by treatment with 1.0 per cent acetic acid and several more washings with distilled water. All the washings, including the sodium carbonate and acetic acid, were poured through the crucible. The crucibles were then dried for 16 hours in an oven at

100° C., and weighed to determine cellulose loss. Uninoculated blanks were carried through the entire procedure, and in all determinations the same lot of cellulose was used.

Although only 20 mg. of cellulose was used per tube, the accuracy of the results obtained with the blank determinations (where recoveries ranged within 0.2–0.5 mg.) seemed to indicate that any loss over 5 per cent would be significant. Actually in only one case was a value as low as 7.0 per cent loss considered as positive. Duplicates tested on the same or on different days gave essentially the same results. The figures showing cellulose loss are too low, as the weight added by bacteria clinging to the cellulose fibers and not removed by washing actually increased the weight of the recovered cellulose.

Under conditions in the rumen, the degree of cellulose decomposition brought about by these bacteria probably is greater than that shown by the *in vitro* tests, where no agitation of the cellulose took place.

Some of the predominating organisms were tested for their ability to utilize urea and to break down cellulose.

Seventeen cultures from dilutions 10^{-10} and 10^{-11} of the rumen contents from a fistulated bull fed a normal diet were tested for cellulose digestion and 12 of these also were studied for their ability to utilize urea. Of these organisms, 15 were rods of varying morphology and two were cocci. All of the cultures tested were found to be Gram-positive, non-motile organisms which produced acid, frequently giving a pH as low as 4.5 in the broth in which they were growing. One coccus and one rod produced slight amounts of gas. Ten out of 12 cultures tested utilized urea and 12 out of 17 broke down cellulose. The majority decomposed 10–20 per cent of the added cellulose, which agreed with the results obtained by Cowles and Rettger (1). One coccus broke down 28 per cent which was higher than any of the rods tested.

Twenty-three cultures isolated from 10^{-10} and 10^{-11} dilutions of rumen contents from sheep fed normal and experimental diets were tested for their ability to utilize urea and break down cellulose. All of them were rods and all of those tested for the following characteristic were Gram-positive organisms. Only one culture was motile. They produced a final pH of about 4.5–5.0 in broth, and only two cultures produced gas. Seven of 17 cultures tested for urea utilization were positive and only 10 out of 23 cultures showed breakdown of cellulose. Again the majority of cultures decomposed 10–20 per cent of the added cellulose, while one broke down 48 per cent.

In agreement with earlier results of Pearson and Smith (9), the analyses to determine whether urea actually was utilized by the organisms showed that 6–21 per cent of the urea had been lost from the broth. The total nitrogen remained the same in all of the tubes, except in two cases, where small amounts of gas had been produced and had escaped.

Because of the small series of tests conducted and differences in diets fed the two species, no conclusions should be drawn about any differences between the species with respect to these two characteristics.

All the samples from fistulated cattle were from one young bull. Although these are preliminary studies, it would seem that the techniques described could be used as a basis for further work in this field.

SUMMARY

1. New techniques for studying the microbiology of the rumen are described. These include a direct slide count technique, a cultural procedure for isolating the predominating flora, a method for obtaining a sample under anaerobic conditions, and methods for the determination of urea utilization and cellulose digestion by the predominating bacteria.

2. The direct slide count technique made use of nigrosine, a negative stain, which reduced to a minimum inaccuracies caused by artifacts.

3. When good cultural conditions prevailed and a fistulated animal was used, slide and cultural counts agreed closely. Material obtained by stomach tube gave consistently lower bacterial counts than samples taken directly from a rumen fistula. To isolate the predominating flora, the most important feature of the cultural technique was the maintenance of anaerobic conditions from the time of sampling until growth was obtained. A rich organic medium with a pH of 6.0 was used and cultures were incubated at 38° C.

4. It was shown by these methods that bacteria are present in the rumen contents of both sheep and cattle in numbers of about 100 billion per gram of fresh material.

5. Bacteria isolated from the highest dilutions of rumen contents from both sheep and cattle were tested for their ability to utilize urea and break down cellulose, and many of these cultures proved capable of performing these functions *in vitro*.

6. A few of the bacteria isolated from the highest dilutions of rumen contents were studied and were found to be mostly Gram-positive, non-motile anaerobic rods. A small number of cocci were cultured.

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MILK LIPASE SYSTEM. I. CHEMICAL ANALYSIS OF FAT, WASHED FAT, AND THE ALCOHOL SOLUBLE FRACTION OF FAT OBTAINED FROM HOMOGENIZED RAW MILK[1]

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Considerable question yet exists as to the specific fatty acids which are involved in lipolysis of milk fat. Davies (2) reports that 60–70 per cent of the increase in the titer of milk fat which results from lipase action is due to oleic acid. On the basis of distillation studies, Hiscox and Harrison (5) and later Gould and Johnson (3) concluded that the fatty acids liberated by lipolysis were largely of the water-insoluble, volatile type. Kelly (8) reported milk lipase to exhibit selectivity by acting more rapidly on the volatile than on the non-volatile substrates.

Only meager attention has been given to the use of chemical analysis of milk fat as a means of ascertaining the effects of lipase action. In a previous study (4), fat from non-rancid and rancid milk was found not to differ appreciably in Reichert-Meissl and Polenske numbers and in the refractive index, even though the rancid fat exhibited a greatly higher acid degree. One limitation of this study, however, was that in the case of the rancid fat, the freed fatty acids remained with the unhydrolyzed portion to be included in the various determinations. Thus, the presence of the free acids may have prevented a true differentiation between the rancid and non-rancid fat by the methods used. In view of this possibility, it seemed desirable to conduct additional studies of chemical changes in milk fat resulting from lipolysis. Procedures were utilized which permit the separation of the free fatty acids from the rancid fat before the fat characteristic determinations are conducted. The results of such studies constitute the basis for this paper.

EXPERIMENTAL PROCEDURE

Mixed-herd milk from the University dairy farm was used in these experiments. Rancidity was produced as desired by homogenizing the raw milk with a low-pressure centrifugal homogenizer and then storing it for several hours at about 35° F. before pasteurizing. The purified fat for subsequent treatment and analyses was obtained from the milk by the standard procedure of separation, churning, and filtration. Filtration was conducted by melting the butter granules and then filtering the butter oil through paper in an incubator maintained at 55° C.

Fat titrations were conducted by the procedure described previously

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(3), and results are expressed as acid degrees (ml. of N NaOH per 100 g. fat). The determinations of all other fat characteristics were made in accordance with official methods of the A.O.A.C. (1).

Two different procedures were used in preparing the fat or its extract for chemical analyses. These are designated as the washing procedure and the alcohol extract procedure.

The washing procedure. This procedure was essentially the same as that described previously (3) as based on the work of Hiscox *et al.* (6), and consists of washing portions of a milk fat–ether mixture with successive lots of weak alkaline solution, removing the retained alkali by washing several times with water, and then drying the fat by means of a hot plate and a 100° C. oven. Various fat characteristics were determined on the fat before and after the washing procedure. One difficulty of this procedure was the susceptibility of the mixture to the formation of an emulsion during the washing with the alkaline solution.

The alcohol extraction procedure. Preliminary trials revealed that an extraction procedure involving hot ethanol for separating the freed fatty acids from the fat could be used with greater efficiency and rapidity than the alkali washing procedure. The following procedure was developed as a result of these preliminary observations.

Sixty grams of butter oil were heated to boiling in 120 ml. of ethanol, the hot mixture centrifuged, and the supernatant ethanol layer largely removed by siphonation. The treatment with hot ethanol was repeated twice, and all of the lots of extractant combined. Following the last centrifuging, the lower fat layer was congealed by placing the container in ice water; then the alcohol layer was decanted.

The alcoholic extracts from two lots of fat were combined, cooled, and held overnight at 32–35° F. A copious white precipitate which formed during this cold storage was removed by filtration in the cold room, and was washed with several portions of cold ethanol at the same temperature. The washings were combined with the filtrate.

The filtered alcohol solution then was heated under a partial vacuum to remove the alcohol. About 30–40 minutes usually were required for removing the alcohol from 120 g. of fat, and the heating temperature generally was under 50° C. The final alcohol extract was subjected briefly to hot plate heating at 100° C. to insure drying and then was held in a desiccator under vacuum until analyzed.

EXPERIMENTAL RESULTS

The alkali washing procedure. The effect of washing rancid and nonrancid fat with the weak alkaline solution is revealed by the data in table 1, which shows changes in acid degree, Reichert-Meissl number, Polenske number, and iodine number (Hanus).

These results show that only in the case of the acid degree is there a distinct relationship between the extent of lipolysis and the change resulting from the alkaline washing. This lowering of the acid degree is to be expected. However, the data do indicate a tendency for the washing procedure to increase the Reichert-Meissl number and decrease the iodine number, but the changes are slight and are no more pronounced for the rancid fats (lots 2, 3, 4) than for the non-rancid fat (lot 1).

On the basis of the results in table 1, the particular procedure used apparently is unsatisfactory as a means of determining the fat changes, other than acidity, which result from lipase action. Because of the failure of the washing procedure to yield satisfactory results, attention was diverted to the alcohol extract procedure.

TABLE 1

The influence of alkaline washing of non-rancid and rancid fat on certain fat characteristics (av. 3 trials)

Lot no.	Treatment of sample	Fat characteristics			
		Acid degree	Reichert-Meissl	Polenske	Iodine (Hanus)
1	Non-washed	0.4	28.4	1.6	36.7
	Washed	0.2	28.7	1.5	36.4
2	Non-washed	3.9	28.7	1.6	36.8
	Washed	0.5	28.7	1.5	36.5
3	Non-washed	4.4	28.3	1.5	36.8
	Washed	0.7	28.6	1.6	36.4
4	Non-washed	4.8	28.3	1.6	36.2
	Washed	0.9	28.7	1.7	36.0

The alcohol-extract procedure—preliminary observations. Before this procedure was devised completely, various preliminary experiments were conducted. One series of these preliminary trials pertained to the efficiency of alcohol in removing the free fatty acids. In this series, 60 g. of rancid fat were heated to boiling in 120 ml. ethanol, centrifuged, and the lower layer of fat congealed in ice water. The top layer, *i.e.*, the alcohol portion, was decanted and titrated. The fat layer then was freed of remaining alcohol by heating on a hot plate, and a sample of the fat removed and titrated. Three additional extractions were made, with titrations of the alcohol extract and the remaining fat being made in each case. Results are presented in table 2.

These data reveal that in the case of this highly rancid fat, three washings removed more than 90 per cent of the free fatty acids as determined by titration of 10-g. samples, and four washings removed more than 95 per cent of these acids. The heat treatment of the fat in the alcohol in itself did not alter the titer, indicating that acidity decreases due to esterification are negligible, if they occur at all.

TABLE 2

The effectiveness of hot ethanol extraction in removing the free fatty acids from butter oil

No. of alcohol extractions	Titer of ethanol extract*	Titer of 10 g. fat samples	Per cent of original acidity in fat
	(*ml. N/20 NaOH*)	(*ml. N/20 NaOH*)	
0		22.9	100.0
1	81.9	10.2	44.5
2	24.0	4.8	21.0
3	7.8	2.1	9.2
4	1.9	1.0	4.4

* Corrected for titer of the alcohol.

Naturally, the alcohol treatment of butter oil not only removes the major portion of the free fatty acids present but also all lipoid material which is soluble in hot alcohol. For example, a 60-g. sample of fat having an acid degree of 11.4, extracted with four successive 120-ml. lots of hot alcohol, showed a total loss in weight of about 17 g., only a portion of which would represent the free fatty acids. Furthermore, observations indicate that only a comparatively small portion of this alcohol-soluble fraction precipitates from the alcohol during overnight storage at 32–35° F. and that this precipitate does not retain an appreciable amount of the free fatty acids. In two typical trials utilizing rancid fat, the clear alcohol solution obtained following overnight storage and filtration required 119.8 and 116.2 ml. of N/10 NaOH, respectively, whereas the corresponding precipitates which were removed by the filtration required but 0.8 and 0.6 ml. N/10 NaOH.

In general, the yield of lipid extract finally obtained for analysis varied directly with the titer of the original fat. For example, three lots of non-rancid fat having an average acid degree of 0.9 yielded an average of 5.8 g. of extract per 100 g. of fat, whereas three lots of rancid fat with an average acid degree of 10.0 yielded an average of 11 g. of extract per 100 g. of original fat.

TABLE 3

Certain chemical characteristics of non-rancid and rancid fat and of their ethanol extracts (av. 3 trials)

Characteristic	Non-rancid			Rancid		
	Fat	Extract	Difference: extract less fat	Fat	Extract	Difference: extract less fat
			(%)			(%)
Acid degree	0.55	5.2	+845.4	10.5	64.5	+514.3
Reichert-Meissl no.	27.8	56.3	+102.5	26.7	43.7	+ 63.7
Polenske no.	1.8	4.1	+127.8	1.7	3.2	+ 88.3
Saponification no.	227.4	249.3	+ 9.6	226.2	238.3	+ 5.3
Iodine no.	35.1	38.3	+ 9.1	35.9	38.3	+ 9.5
Refractive index	1.4544	1.4535	− 0.06	1.4546	1.4528	− 0.1

The extract obtained was a normal golden yellow color and was liquid at room temperature. The flavor of the fat from non-rancid cream was not rancid in flavor, although it retained the flavor of ether to some extent. The extract from the rancid cream possessed an extremely pungent, rancid, bitter flavor.

Several characteristics of non-rancid and rancid fat and their respective alcohol extracts are presented in table 3. These data reveal that in comparison to the original fat, the extracts are higher in acid degree, Reichert-Meissl and Polenske numbers, saponification number, and iodine number, but lower in refractive index. The differences between the characteristics of the fat and its extract are especially pronounced with the acid degree and the Reichert-Meissl and Polenske numbers. In the case of the non-rancid series, the extract (when compared to the original fat) exhibits a two-fold increase in Reichert-Meissl number, a 2.3-fold increase in Polenske number, and a 9.4-fold increase in acid degree. In the rancid series, the acid degree, the Reichert-Meissl, and the Polenske are greater in the extract than in the fat by 6.1-fold, 1.6-fold, and 1.9-fold, respectively. On a percentage basis, the differences between these three characteristics for the fat and extract are less in the rancid series than for the non-rancid series.

The results in table 3 also reveal that the difference in saponification number between the fat and the extract is less for the rancid than for the non-rancid series, whereas the change in iodine number is substantially the same.

DISCUSSION

The results obtained reveal the possibility of utilizing alcohol extraction of milk fat as a simple means of concentrating the fatty acids liberated by lipase action. Also, this alcohol extraction procedure would appear to create conditions more favorable for specific identification of the acids involved since it separates the fatty acids from the major portion of the milk fat.

Even though the alcohol extraction resulted in a portion of milk fat more highly concentrated in free fatty acids than the original fat, the chemical analysis of the extract by the methods used in this study does not appear to yield data sufficiently specific to offer a substantial basis for conclusions relative to the selectivity of the milk lipase system for certain acids or groups of acids. The fat characteristics of the alcohol extracts did reveal a high concentration of the shorter-chained fatty acids, but this occurred in both the non-rancid and rancid series. It is true that on a percentage basis, the concentration of shorter-chained fatty acids did not differ as much between the rancid fat and its extract as between the identical pair in the non-rancid series, whereas the iodine numbers were essentially identical. On this basis, the extract from the rancid fat may be said to contain a higher percentage of saturated, non-volatile, non-soluble fatty acids than the corresponding extract from the non-rancid fat. However, it would appear illogical to draw any definite conclusions in this connection without additional and more specific evidence.

One factor which may affect the results obtained in a chemical analysis of milk fat is the method of preparing the fat. The fat used in this study was prepared by the conventional churning procedure. More recently (7) it has been demonstrated that extracting the fat from milk or cream will yield a product appreciably higher in free fatty acids. Subjecting this extracted fat to chemical analyses may reveal more concerning lipolytic effects than has been achieved in this study on fat obtained by churning.

In general, the use of fat characteristics to distinguish between a non-rancid and a rancid fat apparently leaves much to be desired, even though the free fatty acids present are removed first. However, the failure of the analyses to reveal a high degree of lipase selectivity should not necessarily imply that this is due entirely to a lack of sensitivity in the methods used. More logically, one may accept these results as offering some basis for the assumption that milk lipase acts rather generally on all of the glycerides present and to about the same extent, thus yielding a mixture of free fatty acids which, chemically, resemble quite closely the original fat. It is true, however, that a more specific identification of the fatty acids involved is necessary before final conclusions may be drawn in this connection.

SUMMARY AND CONCLUSIONS

Studies were conducted to ascertain the possibility of determining the specific fatty acids involved in lipase action in milk by applying chemical analyses (a) to milk fat before and after removing the fatty acids by alkaline washing and (b) to an extracted product obtained by treating milk fat with hot alcohol. Acid degree, Reichert-Meissl number, Polenske number, iodine number, and refractive index were used.

Other than in acid degree, the chemical analysis of milk fat before and after the alkali washing did not reveal any distinct difference between non-rancid and rancid fat.

The alcohol extraction method resulted in a fraction in which the free fatty acids were highly concentrated and which also contained a greatly increased proportion of the lower fatty acids in comparison to the original fat. However, the Reichert-Meissl, Polenske, and iodine numbers and the refractive index of the alcohol-soluble fraction were not sufficiently different between the rancid and non-rancid series to reveal any appreciable specificity of the lipase action for particular fatty acids.

A brief discussion is presented of the limitation of the results and of the interpretations which may be placed thereon.

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PHOSPHATASE TEST FOR VARIOUS DAIRY PRODUCTS

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This report describes a laboratory test for various dairy products to determine the adequacy of pasteurization. It includes those modifications needed to produce uniformly quantitative phosphatase values under fixed, standard conditions in applying the test to various common varieties and kinds of cheese and to fluid milk, cream, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk, and cheese whey. The modifications for different products are based on research in the Bureau of Dairy Industry; the results, with the exception of those for Cheddar cheese and fluid milk (1), have not yet been published.

REAGENTS

1. *Buffers:*

a. *Barium borate-hydroxide buffer.* Dissolve 25.0 g. of C.P. barium hydroxide ($Ba(OH)_2 \cdot 8\,H_2O$—fresh, not deteriorated) in distilled water and dilute to 500 ml. In another flask or cylinder dissolve 11.0 g. of C.P. boric acid (H_3BO_3) and dilute to 500 ml. Warm each to 50° C., mix the two together, stir, cool to approximately 20° C., filter, and stopper the filtrate tightly (pH 10.6).

The buffer thus prepared is designated as the 25–11 buffer, the figures indicating the grams per liter of each of the respective reagents. Modifications in the quantities of these two reagents, necessary in preparing the appropriate buffers for testing various products, are indicated in tables 1 and 2.

b. *Color development buffer.* Dissolve 6.0 g. of sodium metaborate ($NaBO_2$)[1] and 20 g. of sodium chloride in water and dilute to 1 l. with water (pH 9.8).

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[1] Obtainable from Amend Drug and Chemical Company, Inc., 117 East 24th Street, New York 10, N. Y.

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TABLE 1

Phosphatase test modifications for different kinds of cheese and cheese of different ages

Kind of cheese	Age or extent of curing; other details	Buffer for opt. pH (9.85–10.20)	Precipitant	Criterion, experimental, phenol equivalent[a]
				γ/0.25 g.
Cheddar, granular, stirred curd, hard cheese	< 1 wk.	25–11[b]	6.0–0.1[c]	3
	1 wk.–1.5 mo.	25–11	6.0[d]	3
	1.5–4 mo.	26–11	6.0	3
	> 4 mo.	27–11	6.0	3
Washed curd, soaked curd, Colby	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–2 mo.	25–11	6.0	3
	> 2 mo.	26–11	6.0	3
Swiss, Gruyère	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–1 mo.	25–11	6.0	3
	1–3 mo.	26–11	6.0	3
	> 3 mo.	27–11	6.0	3
Brick, Muenster	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–1 mo.	25–11	6.0	3
	1–2 mo.	25–11	6.0	3
	> 2 mo.	26–11	6.0	3
Edam, Gouda	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–2 mo.	25–11	6.0	3
	2–4 mo.	26–11	6.0	3
	> 4 mo.	27–11	6.0	3
Blue mold, blue	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–1 mo.	26–11	6.0	3
	1–4.5 mo.	27–11	6.0	3
	> 4.5 mo.	28–11	6.0	3
Camembert, Limburger	< 1 wk.	25–11	6.0–0.1	4
	1 wk.–1 mo.	25–11	6.0	4
	1–2 mo.	26–11	6.0	4
	> 2 mo.	27–11	6.0	4
Monterey	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–2 mo.	25–11	6.0	3
	> 2 mo.	26–11	6.0	3
High moisture Jack	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–2.5 mo.	25–11	6.0	3
	> 2.5 mo.	26–11	6.0	3
Provolone, pasta filata	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–1 mo.	25–11	6.0	3
	1–3 mo.	26–11	6.0	3
	> 3 mo.	27–11	6.0	3
Parmesan, reggiano, monte, modena, Romano, asiago old	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–2 mo.	26–11	6.0	3
	2–6 mo.	27–11	6.0	3
	6 mo.–1 yr.	28–11	6.0	3
	> 1 yr.	29–11	6.0	3
Asiago fresh	Same as Cheddar	...	...	...
Asiago medium	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–1 mo.	25–11	6.0	3
	1–3 mo.	26–11	6.0	3
	> 3 mo.	27–11	6.0	3
Gorgonzola	Same as blue	...	...	...

TABLE 1—(*Continued*)

Kind of cheese	Age or extent of curing; other details	Buffer for opt. pH (9.85–10.20)	Precipitant	Criterion, experimental, phenol equivalent[a]
				γ/0.25 g.
Cottage,[e] cook cheese, koch kaese	Dry	25–11	6.0–0.1	0
	Moist	25–11 (8 + 2)[f]	4.5–0.1	0
Cream cheese	. . .	25–11 (7 + 3)	4.5–0.1	3
Semi-soft cheese	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–1 mo.	25–11	6.0	3
	> 1 mo.	26–11	6.0	3
Soft ripened cheese	< 1 wk.	25–11	6.0–0.1	4
	1 wk.–1 mo.	25–11	6.0	4
	> 1 mo.	26–11	6.0	4
Nokkelost, kuminost, sage cheese	< 1 wk.	25–11	6.0–0.1	3
	1 wk.–1.5 mo.	25–11	6.0	3
	1.5–4 mo.	26–11	6.0	3
	> 4 mo.	27–11	6.0	3
Pasteurized process, past. proc. pimiento, past. proc. with fruits, meats, etc.	Soft, mild	25–11	6.0	3
	Medium firm	26–11	6.0	3
	Firm, sharp (incl. Swiss, Gruyère)	27–11	6.0	3
Past. proc. cheese foods; past. proc. cheese foods with fruits, meats, etc.	Same as pasteurized process			
Past. proc. cheese spreads; past. proc. cheese spreads with fruits, meats, etc.	Soft, high moisture, incl. cream spreads	25–11	6.0	3
	Less soft, incl. Blue	26–11	6.0	3
Cold pack, club; cold pack cheese foods; cold pack cheese foods with fruits, meats, etc.	Mild to medium flavored, soft	26–11	6.0	3
	Sharp, firm	27–11	6.0	3

[a] Values higher than those shown indicate under-pasteurization.

[b] Grams $Ba(OH)_2 \cdot 8\,H_2O$ and H_3BO_3, respectively, per l.

[c] Grams $ZnSO_4 \cdot 7\,H_2O$ and $CuSO_4 \cdot 5\,H_2O$, respectively, per 100 ml.

[d] Grams $ZnSO_4 \cdot 7\,H_2O$ per 100 ml.

[e] See also more sensitive modification in text—alternative.

[f] Eight parts of 25–11 buffer plus 2 parts of water.

c. *Color dilution buffer.* Dilute 100 ml. of color development buffer 1–b to 1 l. with water.

d. *Standard borax buffer,* 0.01-molar, for checking pH meter—pH 9.18 at 25° C.[2] Dissolve 0.9603 g. of pure borax (Bureau of Standards Sample 187) in distilled water (distilled recently or freshly boiled and cooled) and dilute to 250 ml. Keep stoppered tightly.

[2] All pH values reported herein were determined at 25° C. or corrected to that temperature.

2. *Buffer substrates:*

a. *For evaluating pasteurization.* Dissolve 0.10 g. of phenol-free crystalline disodium phenyl phosphate[3] in 100 ml. of the appropriate (tables 1 and 2) barium borate-hydroxide buffer 1–a.

b. *For quantitative results with raw milk and raw-milk products.* Dissolve 0.20 g. of the phenol-free crystalline disodium phenyl phosphate in 100 ml. of the appropriate (tables 1 and 2) barium borate-hydroxide buffer 1–a.

TABLE 2

Phosphatase test modifications for various dairy products other than cheese

Product	Quantity of sample	Buffer for optimum pH (9.85–10.20)	Precipitant	Criterion, experimental, phenol equivalents[a]
Milk:				
Fresh	1 ml.	25–11[b] (5 + 5)[c]	3.0–0.6[d]	2 γ/0.5 ml.
Old or slightly sour	1 ml.	25–11	6.0	2 γ/0.5 ml.
Cream:				
Fresh	1 ml. or 1 g.	25–11 (5 + 5)	3.0–0.6	2 γ/0.5 ml. or 0.5 g.
Old or slightly sour	1 ml. or 1 g.	25–11 (8 + 2)	4.5	2 γ/0.5 ml. or 0.5 g.
Ice cream mix	1 ml.	25–11 (8 + 2)	4.5–0.1	2 γ/0.5 ml.
Sherbet mix	1 ml.	25–11 (5 + 5)	3.0–0.6	2 γ/0.5 ml.
Chocolate drink	1 ml.	25–11 (8 + 2)	4.5–0.1	2 γ/0.5 ml.
Butter	1 g.	18–8	6.0	2 γ0.5 g.
Sweet buttermilk	1 ml.	25–11 (5 + 5)	3.0–0.6	2 γ/0.5 ml.
Cultured buttermilk and fermented drinks:				
Medium acid	1 ml.	25–11	6.0[e]	2 γ/0.5 ml.
Very acid, pH < 4.5	1 ml.	26–11	6.0	2 γ/0.5 ml.
Goats' milk[f]	3 ml.	27–11	7.5–0.1	0 γ/1.5 ml.
Cheese whey	1 ml.	25–11 (5 + 5)	3.0–0.6	2 γ/0.5 ml.

[a] Values higher than those shown indicate under-pasteurization.

[b] Grams $Ba(OH)_2 \cdot 8\,H_2O$ and H_3BO_3, respectively, per l.

[c] Five parts of 25–11 buffer plus 5 parts of water.

[d] Grams $ZnSO_4 \cdot 7\,H_2O$ and $CuSO_4 \cdot 5\,H_2O$, respectively, per 100 ml.

[e] Grams $ZnSO_4 \cdot 7\,H_2O$ per 100 ml.

[f] 4-hour incubation period; use 7.0 ml. of filtrate and add 3.0 ml. of color development buffer 1–b.

3. *Protein precipitants:*

a. *Zinc-copper precipitant for milk.* Dissolve 3.0 g. of zinc sulfate ($ZnSO_4 \cdot 7\,H_2O$) and 0.6 g. of copper sulfate ($CuSO_4 \cdot 5\,H_2O$) in water and dilute to 100 ml. with water. The precipitant thus prepared is designated as the 3.0–0.6 precipitant.

b. *Zinc-copper precipitant for unripened cheese.* Dissolve 6.0 g. of zinc sulfate and 0.1 g. of copper sulfate in water and dilute to 100 ml. with water. This precipitant is designated as the 6.0–0.1 precipitant.

c. *Zinc precipitant for ripened cheese.* Dissolve 6.0 g. of zinc sulfate in water and dilute to 100 ml. with water. This precipitant is designated as the 6.0 precipitant.

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[3] Obtainable, relatively pure, from Applied Research Institute, 2 East 23rd Street, New York 10, N. Y.

The quantities of the respective reagents to use in preparing the precipitants for testing other products, not mentioned under "Protein precipitants" above, are indicated in tables 1 and 2.

4. *BQC or 2,6-dibromoquinonechloroimine solution* (*Gibbs' reagent*): Dissolve 40 mg. of BQC[4] powder in 10 ml. of absolute ethyl or methyl alcohol and transfer to a dark-colored dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

5. *Other reagents:*

a. *Copper sulfate, 0.05 per cent, for standards.* Dissolve 0.05 g. of copper sulfate in water and dilute to 100 ml.

b. *Butyl alcohol.* Specify n-butyl alcohol, boiling point 116–118° C. To adjust the pH, mix 50 ml. of the color development buffer 1–b with a liter of the alcohol.

6. *Phenol standards:*

a. *Stock solution.* Weigh accurately 1.0 g. of pure phenol, transfer to a liter volumetric flask, dilute to a liter with water, and mix. One ml. contains 1 mg. of phenol. Use this stock solution to prepare standard solutions. It is stable for several months in the refrigerator.

b. *Preparation of standards.* Dilute 10.0 ml. of the stock solution 6-a to a liter with water, and mix. One ml. contains 10 γ or *units* of phenol. Use this standard solution to prepare more dilute standard solutions; *e.g.*, dilute 5, 10, 30, and 50 ml. to 100 ml. with water to prepare standard solutions containing 0.5, 1.0, 3.0, and 5.0 γ or units of phenol per ml., respectively. Keep standard solutions in the refrigerator.

In a similar manner, prepare from the stock solution as many more concentrated standard solutions as may be needed, containing, for example, 20, 30, and 40 units per ml.

Measure appropriate quantities of the phenol standard solutions into a series of tubes (preferably graduated at 5.0 and 10.0 ml.) to provide a suitable range of standards as needed, containing 0 (control or blank), 0.5, 1.0, 3.0, 5.0, 10.0, etc., to 30 or 40 units. To increase the brightness of the blue color and improve the stability of the standards, add 1.0 ml. of 0.05 per cent copper sulfate solution 5–a to each.

Add 5.0 ml. of color dilution buffer 1-c and add water to bring the volume to 10.0 ml. Add 4 drops (0.08 ml.) of *BQC* 4, mix, and allow to develop for 30 minutes at room temperature. If the butyl alcohol extraction method is to be used in the test, extract the standards as described under "Conducting the test".

Read the color intensities with a photometer, subtract the value of the blank from the value of each phenol standard, and prepare a standard curve (straight line). When the standards are to be used for visual comparisons, they should be stored in a refrigerator.

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[4] Obtainable from Applied Research Institute.

PHOTOMETRIC DETERMINATION

To read the color in aqueous solution, use a filter with maximum light transmission in the region of 610 $m\mu$ wave length.

To read the color in butyl alcohol, extract the color as described above and centrifuge the sample for 5 minutes to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section 0.25 inch thick from a rubber stopper of suitable diameter to fit in the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of proper size to hold the tube snugly, and insert the double cork section into the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipet with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with a filter with maximum light transmission in the region of 650 $m\mu$ wave length.

If more than approximately 4 ml. of butyl alcohol is required for the photometer used, conduct the test in a larger tube and extract the color, in both the test and the standards, with the necessary quantity of butyl alcohol rather than with 5 ml. specified above.

SAMPLING

1. *Milk and other fluid products:* Mix the product well, pour several milliliters into a small tube, stopper the tube, and keep it in a refrigerator.

2. *Hard cheese:* Take a sample from the interior with a *clean* Roquefort trier, place in a small tube, stopper the tube, and keep it in a refrigerator.

3. *Soft and semi-soft ripened cheese:* Harden the cheese by chilling it in the freezing chamber of a refrigerator. Take special precautions to avoid contaminating the sample with phosphatase that may be present on the surface; use either of the following methods for sampling:

a. Cut a portion from the end of the loaf or from the side of the cheese, extending in at least 2 inches if possible or to a point somewhat beyond the center in the case of a small cheese. Cut a slit about 0.5 inch deep at least halfway around the portion and midway between the top and bottom. Break the portion into two parts, pulling it apart so that it breaks on a line with the slit, being careful not to contaminate the freshly-exposed, broken surface. Remove the sample from the freshly-exposed surface at or near the center of the cheese.

b. Remove the surface of the area to be sampled—*e.g.*, the end and the adjacent sides—with a clean knife or spatula, to a depth of 0.25 inch. Clean the instrument and hands with hot water and phenol-free soap and wipe them dry. Remove the freshly-exposed surface to a similar or greater depth, and repeat the cleaning. Then take the sample from the center of the freshly-exposed area, preferably at or near the center of the cheese in the case of a small cheese.

4. *Process cheese, spreads, butter, and other non-fluid products:* Take the sample from beneath the surface with a clean knife or spatula.

5. *Ice cream and sherbet:* Melt the portion removed and allow it to remain melted for an hour or longer before testing it as a fluid product.

Avoid the use of samples contaminated with mold.

CONDUCTING THE TEST

The chemical principles involved in the detection and measurement of milk phosphatase activity are the same for all dairy products. The fundamental reasons for the general modifications in the test were described earlier (1). Some modifications, described below, have been found necessary for different dairy products, because of their differences in physical properties, compositions, and especially buffering capacities.

Cheese:

Step 1. Weigh, on a *clean* balance pan or watch glass, a 0.50-g. sample (or preferably two samples) and place in a culture tube 16 or 18 × 150 mm. Similarly, weigh another sample and place in a tube as a control or blank. If the cheese is sticky, weigh the sample on a piece of wax paper about 1 × 1 inch and insert the paper with the sample into the tube. Macerate the blank and the test with a glass rod about 8 × 180 mm.

Step 2. Add *to the blank* 1.0 ml. of the appropriate barium buffer 1–a (without substrate added) (table 1), macerate with the rod, leave the rod in the tube, heat for about a minute to at least 85° C. in a beaker of boiling water with the beaker covered so that the entire tube is heated to approximately 85° C., cool to room temperature, and macerate again with the rod.

Step 3. Add *to the test* 1.0 ml. of the appropriate barium buffer substrate 2–a or 2–b (table 1), and macerate.

From this point, treat the blank and the test in a similar manner.

Add 9.0 ml. of the appropriate barium buffer substrate 2–a or 2–b (total, 10.0 ml. added), and mix. The rod may be left in the tube during incubation; if it is removed at this point, cut a piece of filter paper approximately 1 × 1 inch, wrap and hold it tightly around the rod, rotate the rod while withdrawing it from within the tube so as to wipe the rod clean, insert the paper with the adhering fat into the tube, and stopper the tube.

Step 4. Incubate in a water bath at 37–38° C. for 1 hour, mixing or shaking the contents occasionally.

Step 5. Place in a beaker of boiling water for nearly a minute, heating to approximately 85° C. (use a thermometer in another tube containing the same volume of liquid). Cool to room temperature.

Step 6. Pipet in 1.0 ml. of the zinc precipitant 3–c for ripened cheese, or the zinc-copper precipitant 3–b for unripened cheese, and mix thoroughly (pH of mixture, 9.0–9.1).

Step 7. Filter (5-cm. funnel, 9-cm. Whatman no. 42 or no. 2 paper recommended), and collect 5.0 ml. of filtrate in a tube, preferably graduated at 5.0 and 10.0 ml.

Step 8. Add 5.0 ml. of color development buffer 1–b (pH of mixture, 9.3–9.4).

Step 9. Add 4 drops of BQC[5] 4, mix, and allow the color to develop for 30 minutes at room temperature.

Step 10. Determine the amount of blue color by either of two methods:

a. *With a photometer.* Read the color intensity of the blank and that of the test, subtract the reading of the blank from that of the test, and convert the result into phenol equivalents by reference to the standard curve described under "Phenol standards". The butyl alcohol extraction method ordinarily is unnecessary when using a photometer.

b. *With visual standards.* For quantitative results in borderline instances, *e.g.*, tests yielding 0.5 to 5 units of color, extract with butyl alcohol 5–b. Add 5.0 ml. of the alcohol and invert the tube slowly several times; centrifuge if necessary to increase the clearness of the alcohol layer, and compare the blue color with the colors of standards in the alcohol.

With samples yielding more than 5 units, compare the colors in aqueous tests with those of aqueous standards.

Step 11. Dilution method for quantitative results: In tests that are observed to be strongly positive during color development—*e.g.*, 20 units or more—in which 4 drops of BQC may be much less than sufficient to combine with all of the phenol, pipet an appropriate proportion of the contents into another tube, make up to 10.0 ml. with color dilution buffer 1–c, and add 2 drops more of BQC in the case of an unripened product or 4 drops in the case of a ripened product. With each test, dilute and treat the blank in the corresponding manner. Dilute each strongly positive test thus until the final color is within the range of the visual standards or photometer. Allow 30 minutes for color development after the last addition of BQC, and make the reading at the end of the 30-minute period. To correct, multiply by 2 for a 5 + 5 dilution, by 10 for a 1 + 9 dilution, and by 50 for a 1 + 9 followed by a 2 + 8 dilution.

Alternatively, to reduce the amount of yellow off-color, add 2 instead of 4 drops of BQC after each dilution, and allow the color to develop. Then test the completeness of color development by adding a third drop. Repeat the dilution procedure until the addition of an extra drop does not cause any further increase in the amount of blue color.

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[5] For merely detecting under-pasteurization in testing unripened cheese, 2 drops are sufficient, provided the visual standards likewise are prepared with 2 drops.

Step 12. Calculation and evaluation of result: When using 0.5 g. of solid sample and adding a total of 11.0 ml. of liquid, multiply the value of the reading by 1.1 to convert it to units of color or phenol equivalents per 0.25 g. of cheese. If desired, the result may be converted to phenol equivalents per 1 g. by multiplying by 4.4. Evaluate the result by comparing it with the criteria of pasteurization in table 1.

Milk and other fluid products:

Step 1. Pipet a 1.0-ml. sample (preferably two) into a tube and pipet 1.0 ml. into another tube as a control or blank. In testing cream, the sample may be weighed (1.0 g.) if desired; in testing goats' milk, pipet in a 3.0 ml. sample (table 2).

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2", and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml. of the appropriate barium buffer substrate 2–a or 2–b (table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, substituting the appropriate precipitant (table 2) in step 6, and, for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in step 9.

Step 12. Calculation and evaluation of result: When using 1.0 ml. of fluid sample and adding 11.0 ml. of liquid (total liquid 12.0 ml., 5.0 ml. of filtrate used), multiply the value of the reading by 1.2 to convert it to phenol equivalents per 0.5 ml. of sample. If desired, the result may be converted to phenol equivalents per 1 ml. by multiplying by 2.4. Evaluate the result by comparing it with the criteria of pasteurization in table 2.

Butter:

Step 1. Weigh, on a piece of wax paper about 1 × 1 inch, on a balance, a 1.0-g. sample (preferably two) and insert the paper with the sample into the tube. Similarly, weigh another sample and place in a tube as a control or blank.

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2", and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml. of barium buffer substrate 2–a or 2–b (prepared with 18–8 barium buffer, table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, mixing the contents frequently and thoroughly during incubation, substituting the appropriate zinc precipitant (table 2) in step 6, and, for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in step 9.

Step 12. Calculation and evaluation of result: When using 1.0 g. of butter and adding 11.0 ml. of liquid, multiply the value of the reading by

1.1 to convert the result to phenol equivalents per 0.5 g. of butter. Evaluate the result by comparing it with the criterion of pasteurization in table 2.

MODIFICATIONS FOR DIFFERENT CHEESES AND OTHER DAIRY PRODUCTS

Different dairy products, and different kinds of cheese and cheeses of different ages, have different buffering capacities, and, therefore, some of them require modification of concentrations of the reagents. The modifications of the barium buffer needed to produce optimal pH conditions during incubation (9.85–10.20), and of the precipitant to yield uniformly clear filtrates and to minimize interference during color development under optimal pH conditions (9.3–9.4), are specified in tables 1 and 2.

With some samples, especially cheese samples of unknown history, slight deviations from the optimal pH range may occur, but such deviations do not very materially affect the results. For example, pH values as low as 9.6 or as high as 10.35 during incubation have been found to result in an average decrease of not more than 20 per cent below the maximum in the quantity of phenol liberated. The use of the 25–11 buffer substrate with samples for which the 27–11 buffer substrate is specified yields pH values not lower than 9.8.

A trace of cloudiness in the filtrate, following the use of the precipitant as prescribed, indicates that the concentration of barium hydroxide in the buffer was not sufficiently great, *i.e.*, that the buffer substrate was not sufficiently alkaline. For example, the 25–11 buffer diluted 5 + 5 with water, for use with fresh milk, may yield a cloudy filtrate if used with old milk having a pH below approximately 6.0, or with milk that has soured, and the test should be repeated with a more concentrated buffer and precipitant. Likewise, the 25–11 buffer, for use with unripened cheese, may yield a cloudy filtrate if used with ripened cheese, indicating that the concentration of the buffer used was not sufficient. Increasing the concentration of zinc sulfate in the precipitant also eliminates turbidity of the filtrate.

In testing cheese of unknown history or age, information as to the percentage of solids, especially the nonfat solids, is useful as an indication of the correct buffer to use; cheese with a relatively high percentage of nonfat solids generally requires the use of a relatively concentrated buffer to adjust the pH of the mixture correctly.

For precise quantitative results on unknown samples, adjust the pH to 10.0–10.05 for the incubation.

Cottage cheese curd is heated in the presence of considerable acid during manufacture, and therefore its phosphatase values are comparatively low. Alternatively, to increase the sensitivity of the test on cottage cheese, apply the following modifications: Use a 1.0-g. sample, 27–11 buffer substrate, 2-hour incubation, 6.0–0.1 precipitant, and a pasteurization criterion of 2 units per 0.5 g.

Phosphatase activity is much less in goats' milk than in cows' milk. The details of a modification designed to increase the sensitivity of the test applied to goats' milk are specified in table 2.

To test concentrated milk products, reconstitute the product with water to its original concentration of milk solids and test in the manner specified for the original product.

In this work, phosphatase activity caused by microorganisms has not been found in any fresh or reasonably fresh products. It has been encountered in some samples of old butter and old cream, on the surfaces of some soft and semi-soft ripened cheeses, and in several specific cultures of microorganisms. Such activity, in all samples tested to date, is indicated by blue color in the controls or blanks heated as described above. This seems to indicate, as others have pointed out earlier in tests on a few strains of microorganisms, that microbial phosphatases require a higher temperature for inactivation than milk phosphatase. If any blue color is noted in the blank, the presence of microbial phosphatase should be suspected. To test further for the presence of microbial phosphatase, repeat the determination, adding 1 ml. of the appropriate barium buffer (without substrate) to the blank and heat it for 5 minutes in boiling water in a covered beaker. If the blank treated thus is negative, it indicates that the blue color in the original blank was due to microbial phosphatase, *i.e.*, a "false positive" sample.

PRECAUTIONS

The length of time that the crystalline disodium phenyl phosphate and the BQC powder will remain stable can be increased greatly by keeping them in the freezing chamber of a refrigerator.

The glassware, stoppers, and sampling tools should be scrupulously clean, and it is desirable to soak them in hot, running water after cleaning.

The solid barium hydroxide and the barium buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

Phenolic contamination from plastic closures on reagent bottles has been encountered, and therefore the use of plastic closures should be avoided. Rubber stoppers should not be used in flasks in which butyl alcohol is stored. Glass or cork stoppers should be used.

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EFFECT OF HAY FEEDING IN SUMMER ON MILK PRODUCTION AND GRAZING PERFORMANCE OF DAIRY COWS

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Previous studies in Louisiana (1, 2, 3) showed that milk cows apparently suffer from summer daytime atmospheric conditions. As a result, on warm days the grazing time between morning and evening milkings averaged less than 2 hours daily. While not grazing the cows were found in the shade. This time spent in the shade frequently was for periods of from 5 to 6 hours. During these periods cows did not have opportunity to refill their paunches unless they ventured into the sunshine, and this they did not do.

The present study was initiated in an effort to determine the value of providing hay as a supplemental midday feed. Extra feed thus would be provided to the animals at a time about midway between the time cows ceased grazing in the forenoon and when they entered the milking barn in the evening. Not only was there a desire to learn how this supplemental feeding would affect milk production, but information also was sought on how such a practice might change the grazing performance of cows and their reactions to heat. Unpublished data from a preliminary Louisiana trial conducted in 1940 gave non-significant increases in milk yields when hay was fed at milking time during summer months while cows were on pasture. The hay fed was white Dutch clover and averaged 9 lb. daily. In that trial no check was made on possible changes in body weight of cows caused by the hay feeding.

EXPERIMENTAL PROCEDURE

Twenty cows, consisting of four groups of five Holstein cows each, were used in a manner appropriate for two double-reversal experiments. Cows were assigned to the groups so as to secure as much uniformity as possible, considering milk yield, stage of lactation, and other factors. Milk yields averaged approximately 27 lb. per cow daily at the start of the experiment. The experiment continued for 60 days, being divided into three 20-day periods. The first 10 days of each period were considered as the transition or preliminary period, and only the last 10 days were utilized in measuring results.

During four relatively clear, warm days of each test period cows were watched continuously, day and night, in order to secure a record of their grazing performance.

Hay fed in the trial was that cut in May, 1946, from a white Dutch clover field in St. Mary Parish, Louisiana. About two-thirds of the hay consisted of clover, while the remainder was grass, which consisted primarily of canary

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grasses (*Phalaris agusta* Ness and *Phalaris caroliniana* Walt). This grass portion was coarse and stemmy and was low in palatability.

During each of the three periods two groups of cows served as checks and received no hay. A third group was fed hay free-choice at the rate of 0.5 per cent of body weight per cow; the fourth group was fed in a similar manner all the hay they would clean up. The hay was placed in feeding racks located in the shade within pastures at around 11:30 a.m. daily, and cows ate most of their hay prior to entering the milking barn at 3:30 p.m. Prior to each milking, an appropriate grain mixture was fed to cows, individually, at a daily rate of 0.4 lb. for each pound of milk produced in excess of 13 lb. daily.

Excellent pasture was available to the cows throughout the experiment. More than average amounts of rainfall caused a greater than usual growth of the prevailing grasses—carpet (*Axonopus affinis*), Bermuda (*Cynodon dactylon*), and Dallis (*Paspalum dilatatum*). The pasture area was divided by electric fencing so that the ten cows in check groups grazed one-half the area and each of the hay-fed groups of five cows each grazed one-fourth of the pasture.

Cows were weighed for 3 consecutive days at the start and end of each experimental period in order to determine changes in body weight.

RESULTS

Hay consumption. Actual amounts of hay consumed after deducting that refused averaged 5.54 lb. per cow daily under the full-feeding schedule and 4.69 lb. per cow when limited to 0.5 per cent of body weight. At the time the experiment was planned it was estimated that hay consumption under the full-feeding plan would be twice that on the restricted level of 0.5 per cent of body weight. The fact that an average of only 18.1 per cent more hay was consumed was due partially to the presence of low-quality canary grass in the mixed hay fed.

Milk production and fat test. Milk yields (table 1) based on averages for the various groups and levels of hay feeding showed only minor changes due to hay feeding. Group A averaged 0.4 lb. more milk per cow daily on limited hay than during the check period, but group B averaged 1.0 lb. less per cow daily on limited hay than when on check periods. On full feeding of hay group C averaged 0.1 lb. per cow daily less than during the check period, and group D had an advantage in favor of hay feeding of only 0.1 lb. of milk per cow daily.

An analysis of fat tests for individual cows during the experiment revealed no uniform trend either upward or downward attributable to hay feeding. Most of the variation found in tests was between periods, affecting in similar manner cows fed hay and those without hay.

Day and night grazing time. The feeding of hay had only a minor

TABLE 1

Effect of hay feeding at two levels on daily milk yield per cow

Cow groups	Av. milk yields on:			
	No hay	Limited hay	Full-fed hay	Difference
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
A	22.5	22.9*		+ 0.4
B	22.6*	21.6		- 1.0
C	25.4		25.3*	- 0.1
D	23.9*		24.0	+ 0.1

* These averages represent mean values for 5 cows during first and third test periods. All other averages are based on those for 5 cows during second test period.

effect on grazing time (table 2). Limited hay feeding apparently caused no reduction in grazing time; full-hay feeding resulted in less time spent grazing. Daytime grazing (between morning and evening milkings) averaged 1.92 hours for check periods and 1.63 hours for full-hay feeding periods. Night grazing (between evening and morning milkings) averaged 4.85 hours for check periods and 4.47 hours under full-hay feeding periods. The 24-hour totals for grazing thus averaged 6.77 hours for checks and 6.1 hours for full-hay feeding. The indicated reduction in grazing time due to the full feeding of hay was 0.67 hour or 40 minutes.

A more detailed breakdown of the grazing data by periods (table 3) reveals trends previously reported for Louisiana milking cows (1, 2) plus added information on activities of cows other than grazing. Daytime graz-

TABLE 2

Effect of hay feeding on day and night grazing time among groups of cows

Treatment	Periods				
	I (*hr.*)	II (*hr.*)	III (*hr.*)	Av. (*hr.*)	
	(Daytime grazing)				
Limited hay	2.53	1.58	2.22	2.12	
Full hay	2.20	0.97	1.73	1.63	
Check	2.38	1.17	2.15	1.90	
Check	2.58	0.85	2.35	1.93	
Av.	2.47	1.13	2.12	1.90	
	(Night grazing)				
				Av.	24-hr. av.
Limited hay	4.40	4.15	5.30	4.62	6.73
Full hay	4.42	4.10	4.88	4.47	6.10
Check	4.25	5.15	4.97	4.78	6.68
Check	4.30	5.30	5.17	4.92	6.85
Av.	4.35	4.67	5.07	4.70	6.60

TABLE 3

Pasture performance of Holstein cows (20 cows with observation for 3 days during each period)

Daytime summary

Periods	Air temp.†	Grazing			Not grazing			
		In sun	In shade	Total	In shade not in mud	Lying in mud	Eating hay*	Total
	(°*F.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)
I	81	1.99	0.44	2.43	3.45	1.25	0.80	5.10
II	91	0.72	0.42	1.14	4.10	1.76	1.00	6.86
III	89	1.76	0.36	2.12	4.74	0.08	1.18	6.00
Av.	87	1.49	0.40	1.89	4.10	1.03	0.66	5.99

Night summary

Periods	Air temp.‡	Grazing	Not grazing		
		Total	Standing	Lying down	Total
	(°*F.*)	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)
I	76	4.36	1.33	3.89	5.22
II	79	4.67	1.89	2.83	4.72
III	72	5.08	0.63	3.84	4.47
Av.	76	4.70	1.28	3.52	4.80

24-hr. totals

	Total time in pasture	Grazing		Not grazing	
	(*hr.*)	(*hr.*)	(%)	(*hr.*)	(%)
Daytime	8.13	1.90	23.3	6.23	76.6
Night	9.50	4.70	49.5	4.80	50.5
Total	17.63	6.60	37.4	11.03	62.6

* Only one-half of cows were fed hay during each period, so time spent eating hay is for one-half of cows.

† At 3: 00 p.m.

‡ At 11: 30 p.m.

ing averaged 1.49 hours in the sunshine and 0.4 hour in the shade of trees, etc. The most daytime grazing occurred during the first period, when air temperatures were the lowest (81° F. at 3:00 p.m.). During this period grazing averaged 2.43 hours as compared to 1.14 hours for the second period when air temperatures at 3:00 p.m. were 91° F.

Daytime spent not grazing consisted of 4.1 hours standing or lying in the shade (not in mud), 1.03 hours lying in mud, and 0.66 hour eating hay (for those fed hay). Grazing at night averaged 4.7 hours, while time not grazing consisted of 1.28 hours with cows standing and 3.52 hours with them lying down.

Body weight changes. Cows, on an average, lost weight during each of the first two 20-day periods and gained during the third period (table 4).

TABLE 4

Changes in body weight during experimental periods

	Av. changes in body weights for period:		
	I	II	III
	(*lb.*)	(*lb.*)	(*lb.*)
A. Check group	− 1	−20	+17
B. Check group	−26	− 6	+17
C. Limited hay group	− 7	−13	+16
D. Full-fed hay group	+ 8	−17	+17

There was no uniform trend indicating that hay feeding aided in maintaining body weight. The fact that cows in all groups gained rather than lost in body weight during the third period may have been due to the more favorable weather or better pasture, or to a combination of these two factors.

SUMMARY

1. Two double-reversal trials involving four groups of five cows each were conducted simultaneously over a period of three 20-day periods. The effect of feeding limited and full feedings of hay to cows at midday while they were in the shade was tested while cows had access to excellent permanent pasture. The fact that the hay fed consisted of one-third grass (and two-thirds clover) may have caused the results to be quite different from those which might have been secured had all of the hay consisted of high quality clover.

2. Average change in production for the two groups used in determining the influence of limited hay feeding (0.5 per cent of body weight daily) was +0.4 and −1.0 lb. of milk per cow daily. Daily hay consumption averaged 4.69 lb. per cow during periods fed hay.

3. The two groups on the full-hay feeding phase showed changes due to the hay supplementation averaging +0.1 and −0.1 lb. per cow daily compared to check periods, and during periods fed hay consumed an average of 5.54 lb. per cow daily. Changes in milk yields due to feeding of hay proved insignificant and economically unprofitable.

4. No reduction for time spent grazing due to limited hay feeding could be found, although those on full feeding of hay grazed an average of 40 minutes per day less than during check periods without hay. This difference appeared to be due to chance variation rather than definitely to be caused by hay feeding.

5. Hay feeding failed to produce definite changes in body weights of cows other than those also shown by check groups not fed hay.

6. Grazing in daytime consisted of 1.49 hours in the sunshine and 0.4 hour in the shade. Time during day not grazing averaged 4.1 hours with

cows standing or lying in shade, 1.03 hours lying in mud, and, for periods fed hay, 0.66 hour eating hay.

7. Activities at night averaged 4.7 hours actually grazing, 1.28 hours standing not grazing, and 3.52 hours lying down.

CONCLUSIONS

Results of this experiment would indicate that cows on very good pasture under conditions existing in southern Louisiana benefit only slightly if at all from the feeding of hay at midday. Hay feeding reduced little, if any, the time that cows actually graze either in daytime or night. Had better quality hay been used, it is possible that there may have been consistent beneficial effects from the hay feeding and a greater influence on grazing time.

The information secured confirms previous work in Louisiana (1) on how grazing time is shortened by increases in air temperature, and that cows in summer graze much more at night than in the daytime. The tendency for Holstein cows to lie in mud when possible again was noted (2). It remains for future experimentation to determine the practicability of hay supplementation to cows when on poor pasture and whether or not supplemental feeds other than hay might produce better results than those secured in this experiment.

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THE CONCENTRATION AND OUTPUT OF CAROTENOID PIGMENTS, VITAMIN A, AND RIBOFLAVIN IN THE COLOSTRUM AND MILK OF DAIRY COWS

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The importance of vitamin A in the nutrition of the newborn calf is well established. Recent work at the University of Illinois (15) has demonstrated that when young calves (less than 48 hours old) are placed on a riboflavin-free synthetic diet, a deficiency syndrome which can be cured by the administration of riboflavin develops in about 2 weeks. Thus, the importance of the riboflavin content of colostrum and milk in the nutrition of the very young calf is emphasized.

Likewise, the data on the carotene and vitamin A content of colostrum are numerous, while much fewer data are available on riboflavin.

Since the work of Dann in 1933 (2), a substantial number of papers have appeared in which data on the vitamin-A value of colostrum were presented, notably those of Semb *et al.* (9), Stewart and McCallum (11, 12), Henry *et al.* (5), Kramer *et al.* (6), Sutton *et al.* (13), Hansen *et al.* (4), and Spielman *et al.* (10). In some of these reports the colostrum obtained from two or more milkings was combined for assay, and in many instances the sampling procedure was not described. In the experience of the authors the method of sampling is important in obtaining a sample for an accurate estimation of the vitamin A and carotene potency of the colostrum obtained at any particular milking.

The data on the riboflavin content of colostrum are not so numerous. In 1938 Kramer *et al.* (7) made a biological determination of the riboflavin in the colostrum and milk of two Holstein and two Jersey cows on the first, fifth, and thirtieth day of lactation. They found that first-day colostrum was approximately two times as potent in riboflavin as fifth-day milk and three times as potent as thirtieth-day milk.

Theophilus and Stamberg (14) made fluorometric determinations of the riboflavin content of the colostrum and milk of two Holstein and two Jersey cows on each of 8 consecutive days following parturition and on the twentieth day. They found that the colostrum was from 2.5 to 3.5 times as high in riboflavin as normal milk. Pearson and Darnell (8), using a larger number of cows of the Holstein and Jersey breeds, found that colostrum at parturition contained an average of 6.10 μg. of riboflavin per ml. and was approximately 3.4 times as potent as normal milk.

The data on riboflavin content of cows' colostrum which have appeared have been from the Jersey and Holstein breeds. While the decline from

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day to day has been reported by Pearson and Darnell (8), the decline with successive milkings has not been reported. Only limited data are available on the total output of carotene, vitamin A, and riboflavin during the early stages of lactation.

EXPERIMENTAL PROCEDURE

In an experiment designed to evaluate the use of all colostrum produced in the raising of dairy cows, the calves were not allowed to nurse following birth. Thus samples for carotene, vitamin A, and riboflavin estimation which were truly representative of each milking were obtained. Oxytocin injections, as used by the Wisconsin workers (4), were not used to evacuate completely the mammary gland. The calves were left in the box-stalls with their dams for the first 3 days, but tied so they could not nurse. This procedure has been found by the Wisconsin group to be essential for a normal "let-down" of milk for the first few milkings following parturition.

The cows were milked twice daily and the complete milkings were weighed and sampled for assay. Data for the first ten milkings and the twentieth milking are reported in this paper. The assay samples were placed in brown glass bottles and refrigerated until analyzed, which usually was within 12 hours from the time of milking.

The cows used for this study were not selected, but the analyses were made on the colostrum and milk of all the cows and heifers of the Ohio State University herd which freshened during the 1946 pasture feeding period.

Vitamin A and total carotenoid pigments were determined by the method of Boyer *et al.* (1) and riboflavin by the method of Hand (3). Quantitative modifications were made in both procedures to adjust for the high potency of the colostrum.

RESULTS AND DISCUSSION

The data for carotene, vitamin A, and riboflavin content of colostrum and milk are summarized in tables 1, 2, and 3, respectively. The average output of these constituents in the colostrum and milk of these cows was calculated and is presented in table 4. In calculating the output of vitamin-A activity in terms of International Units, 1 mg. of vitamin A was considered equivalent to 4,000 I.U.

The data for individual cows show considerable variation for all the constituents studied, the greatest variation occurring in the first milking. For example, the riboflavin content varied between 2.7 and 11.5 mg. per l., carotene between 0.77 and 11.81 mg. per l., and vitamin A between 0.85 and 11.25 mg. per l. for the first milking.

Much of this variation cannot be accounted for at present. Although the cows were on pasture and were fed and managed similarly during the time these data were obtained, not all freshened at the same time, and perhaps some of the difference between individuals was due to cumulative effects

TABLE 1

The carotene content of cows' colostrum and milk

Breed	No. of cows	Carotene mg./l. at successive milkings										
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	20th
Ayrshire	6	3.73	3.39	1.72	1.23	1.02	0.58	0.63	0.38	0.42	0.37	0.25
Guernsey	8	8.64	4.72	2.60	1.70	1.29	1.02	0.89	0.64	0.67	0.63	0.52
Holstein	9	2.89	1.73	1.13	0.98	0.85	0.57	0.42	0.42	0.41	0.35	0.30
Jersey	7	3.35	2.88	1.80	1.85	1.48	1.55	0.93	0.65	0.60	0.52	0.43
Brown Swiss	5	4.97	4.20	2.09	1.09	1.09	0.87	0.66	0.53	0.41	0.39	0.41
All breeds	35	4.73	3.29	1.82	1.36	1.13	0.90	0.69	0.57	0.52	0.46	0.41

TABLE 2

The vitamin A content of cows' colostrum and milk

Breed	No. of cows	Vitamin A mg./l. at successive milkings										
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	20th
Ayrshire	6	1.82	2.12	1.17	0.89	0.74	0.42	0.48	0.39	0.36	0.38	0.48
Guernsey	8	2.79	1.29	0.86	0.55	0.40	0.33	0.32	0.27	0.26	0.27	0.21
Holstein	9	1.69	1.29	0.75	0.73	0.68	0.49	0.42	0.45	0.45	0.42	0.45
Jersey	7	1.44	1.36	1.05	0.97	0.97	0.84	0.68	0.44	0.44	0.39	0.52
Brown Swiss	5	3.48	2.47	1.20	0.98	0.70	0.54	0.44	0.39	0.35	0.27	0.51
All breeds	35	2.40	1.62	0.96	0.79	0.68	0.51	0.46	0.38	0.37	0.35	0.40

TABLE 3

The riboflavin content of cows' colostrum and milk

Breed	No. of cows	Riboflavin mg./l. at successive milkings										
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	20th
Ayrshire	6	6.95	4.05	2.64	2.13	1.96	1.90	1.85	1.82	1.64	1.60	1.54
Guernsey	8	7.38	4.58	2.90	2.65	2.80	2.75	2.65	2.60	2.58	2.52	2.47
Holstein	9	4.83	2.71	1.85	1.80	1.76	1.73	1.66	1.44	1.42	1.44	1.47
Jersey	7	6.34	4.00	3.21	2.87	2.64	2.33	2.05	2.26	2.24	2.16	1.95
Brown Swiss	5	5.52	3.00	1.74	1.74	1.70	1.68	1.60	1.67	1.82	1.82	1.56
All breeds	35	6.15	3.66	2.44	2.25	2.20	2.10	1.98	2.01	1.96	1.95	1.83

TABLE 4

The average output of carotene, vitamin A, vitamin-A activity in International Units, and riboflavin in cows' colostrum and milk

Constituent	Av. output at successive milkings										
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	20th
Carotene, mg.	26.77	11.23	9.11	9.32	7.55	5.97	5.00	4.02	3.81	3.54	3.74
Vitamin A, mg.	11.94	7.51	5.05	5.16	4.48	3.46	3.43	2.70	2.67	2.45	3.67
Vitamin A, I.U.	93,370	48,750	35,380	36,180	30,500	23,790	22,050	17,500	17,030	15,400	20,790
Riboflavin, mg.	33.1	12.6	12.2	14.8	14.5	15.1	14.5	13.7	14.6	14.3	16.0
Total milk, lb.	12.7	8.3	12.7	15.0	16.0	16.6	16.7	16.1	17.1	17.9	19.9

of continued pasture feeding. The length of the dry period also varied among the individual cows. Further data are being obtained for a statistical study of the effects of the length of the dry period on colostrum composition. The potency and total output of vitamin A in International Units were no higher in these cows than that previously reported from this laboratory (13) for cows that freshened in the winter months.

The decline in potency with successive milkings during the early stages of lactation is striking. A few of the cows were milked out incompletely at the first milking due to severe udder congestion or for other reasons. In each of these cases the second milking was higher in carotene and vitamin A content. This observation is in agreement with the work of Hansen *et al.* (4). The potency for riboflavin did not follow the carotene and vitamin A in these cases that were incompletely milked at the first milking. In no instance was riboflavin more concentrated in the colostrum of the second milking.

When cows are completely milked out at the first milking following parturition, the quantity of colostrum obtained at the second milking 12 hours later is significantly less (table 4). This fact, coupled with the reduction in potency, accounts for a drastic decrease in total output of the constituents studied.

In substantiation of the work of Theophilus and Stamberg (14), a breed difference in riboflavin potency of colostrum and milk is evident. The average riboflavin potency of the first milking found in this study is almost identical with that reported by Pearson and Darnell (8). The carotene data also support the observation that Guernsey colostrum has a higher concentration of this constituent than that from other breeds studied (9).

The high potency of colostrum in vitamin-A activity and riboflavin, both important dietary essentials for the newborn calf, emphasizes the practical importance of using all the colostrum produced in the raising of herd replacements.

SUMMARY

The potency and output of carotene, vitamin A, and riboflavin in the colostrum and milk of 35 pasture-fed cows representing five dairy cattle breeds were determined for the first ten milkings and the twentieth milking. Cows of all ages were included.

The data obtained show a rapid decline in the concentration of these constituents with successive milkings. A level closely approaching that of normal milk is reached by the end of the third day following parturition (sixth milking).

These data show first milking colostrum to be approximately ten times as potent in carotene, six times as potent in vitamin A, and 3.3 times as potent in riboflavin as normal milk obtained at the twentieth milking.

The practical importance of using all the colostrum produced in the raising of calves is pointed out.

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THE INFLUENCE OF MANAGEMENT, BREED, AND SEASON UPON THE pH OF BULL SEMEN[1]

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The fact that spermatozoan viability is limited to a narrow pH range has emphasized the need for the consideration of hydrogen-ion concentration in semen studies. However, little investigation has been conducted to determine the relationship of pH to other pertinent data on semen characteristics.

Webster (10) first reported that the hydrogen-ion concentration of bull semen varied from pH 7.0 to 7.5. Milovanov's early observations, quoted by Anderson (1, p. 4), showed bull semen to be slightly acid, with the pH usually ranging from 6.5 to 6.8, but in one case as low as 5.5. He further stated that the presence of abnormal amounts of accessory gland secretion may be responsible for an alkaline reaction (pH 7.0 to 7.5). Davis (2) found that collection techniques modified the hydrogen-ion concentration of resultant samples to a marked degree. Rectal massage of the ampulla and the accessory organs yielded semen of pH 7.5 to 8.0, while the use of the artificial vagina produced semen whose pH usually was below 7.0.

Sergin (8) reported an average pH of 6.74 when using a quinhydrone electrode for the determination. Anderson (1, p. 4), from a study of 11 fertile bulls, reported the mean pH of semen to be 6.73 ± 0.02. He further observed that, in conditions involving pathology of the genitalia, the reaction was characteristically alkaline. This observation was constant in hypogonadism and in epididymitis. Where consecutive samples had been studied, he stated that the second ejaculate was more acid and was of better quality than the first. However, on 571 samples from 11 bulls studied by Davis and Williams (3), the mean pH of the first ejaculate was 6.85 and that of the second ejaculate 7.23.

Schneerson (7) showed that sperm concentrations up to 1.8 billion per milliliter existed in ejaculates having a pH of 5.8 to 6.0, while samples at pH 7.0 contained an average of only 0.6 billion per milliliter. Anderson (1, p. 4) reported a similar observation.

The relationship between a long survival time and a more acid pH has been described by Schneerson (7). Dougherty and Ewalt (4) have shown a correlation between pH change during storage and viability of the spermatozoa.

EXPERIMENTAL PROCEDURE

Observations were made on a series of semen samples collected at bimonthly intervals during the early part of the study and at alternate

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monthly intervals for the latter part. A total of 371 ejaculates from 66 bulls housed at five different studs was examined from August, 1945, through June, 1946, a period which included the heaviest breeding season.

These observations presented an opportunity to investigate additional relationships between semen pH and such factors as seasonal and other environmental conditions. Anderson (1, p. 137) had shown such conditions to have definite effects upon other semen characteristics and upon the resultant conception rate.

The accumulated data were examined by an analysis of variance with the view to answering such questions as: (a) Are environmental conditions reflected in pH of semen? (b) Are within-bull and within-breed differences evident in pH of semen? (c) Are seasonal changes noticeable in pH of semen? (d) Are there significant changes in the pH between first and second ejaculates?

RESULTS

Table 1 shows the mean pH readings of the semen used in this study. Since there were striking extremes in both care and management of the different bull studs, a question immediately raised was whether such practices might be manifest by their effect upon the pH of the semen. The significant variations ($P \leqq 0.01$) of pH readings between studs (table 2, no. 1) indicated a positive relationship between the semen pH level and the individual studs.

TABLE 1

Mean pH readings of 1st and 2nd ejaculations by breed and place

Stud no.	Ejaculation no.	Brown Swiss (10 bulls)	Guernsey (14 bulls)	Holstein (21 bulls)	Milking Shorthorn (14 bulls)	Mean pH of stud
1	1st	6.61	6.87	6.65	6.77	6.73
	2nd	7.56	7.39	6.68	6.95	7.15
2	1st	6.54	6.63	6.38	6.57	6.53
	2nd	6.68	6.71	6.47	6.61	6.62
3	1st	6.55	6.67	6.62	...	6.61
	2nd	6.61	6.64	6.71	...	6.65
4	1st	...	7.14	7.04	7.49	7.22
	2nd	...	7.05	6.94	7.15	7.05
5	1st	6.67	6.62	6.60	6.88	6.69
	2nd	6.82	6.74	6.69	7.04	6.82
Av.	1st	6.59	6.79	6.66	6.93	6.76
	2nd	6.92	6.91	6.70	6.94	6.86

A highly significant relationship (table 2, no. 3) existed between the individual studs and the pH of the first and second ejaculates from their bulls. This was seen as a greater shift in pH from the first to the second ejaculations within one stud as compared to a relatively small change between the pH of the two ejaculates at another stud.

A positive correlation ($P \leqq 0.05$) was found to exist within breeds of the four dairy breeds investigated (table 2, no. 4). The application of such a relationship might well be considered in the preparation of semen substrates. Phillips *et al.* (6) have shown significant relationships of other semen characteristics to breed.

TABLE 2

Results from an analysis of variance made on 371 semen samples

Source of variation	Degrees of freedom	Sums of squares	Mean squares
1. Places	4	9.8117	2.452*
2. Order of ejaculation	1	0.2675	0.265
3. Order × place	4	1.0158	0.254*
4. Breeds within place	14	3.1086	0.222†
5. Order × bulls within place	14	0.7045	0.050
6. Bulls within breed and place	40	4.4964	0.112†
7. Order × bulls within breed and place	40	2.6372	0.066
8. Days within bulls	71	5.1127	0.072†
9. Order × days within bulls	71	3.1830	0.045

* Signifies $P \leq 0.01$.
† Signifies $P \leq 0.05$.

The tendency of individual animals to maintain a significant level ($P \leqq 0.05$) in their semen pH also was noted (table 2, no. 6). Repeated samplings from 21 bulls (six to twelve ejaculations per bull) were used for this determination.

Finally, the effect of seasons upon pH (table 2, no. 8) was significant at the 5 per cent level and verified the findings of Swanson and Herman (9).

DISCUSSION

Possibly of greatest interest from a practical viewpoint was the relationship which appeared between the semen pH from a stud and the performance of that stud. Stud no. 4, with the bulls having the highest mean pH (7.13), has been disbanded because of its inability to maintain a normal conception rate. Stud no. 1, with the next highest mean pH (6.93), has been reorganized completely. Stud no. 2, showing the lowest mean pH (6.57), is the only one of the five which has remained relatively unchanged.

One immediately would suspect a possible effect of feeding and management practices upon semen pH. Practices in stud no. 4 (table 1) were the antithesis of most good husbandry methods. The bulls were cared for poorly, were kept in an ill-ventilated barn, and never were exercised except when removed from the tie stalls for semen collections. The feet of the bulls were in extremely bad shape, with overgrown claws resulting in permanent disability of several of the animals. The feed was of a low protein content. Timothy hay and Reed canary grass hay were the only roughages fed. The concentrate mixture was fed irregularly, with interruptions ranging from 1 to 6 weeks in length.

In contrast was the stud having the lowest mean pH. The barn was clean, dry, and free from strong urine odors. The bulls were exercised regularly and groomed daily. The feet were kept properly trimmed. Feeding practices were in accord with those widely advocated, and A and D vitamin supplements were included in the ration.

Of further interest was the apparent relationship of mean pH to breed of bull, for the pH readings of the semen from Holstein and Brown Swiss bulls were relatively lower than those of the other breeds studied. However, as one considers other variations that have been noted between breeds (such as differing conception rates, lengths of active service and libido), the feasibility of this relationship can be recognized more easily. The relationship needs further study, since its application to diluent preparations of standardized pH may be of importance. One might speculate that, with advancing knowledge of semen substrates, will come the preparation of diluents with pH standardized to individual breeds, possibly enhancing semen longevity and conception rate.

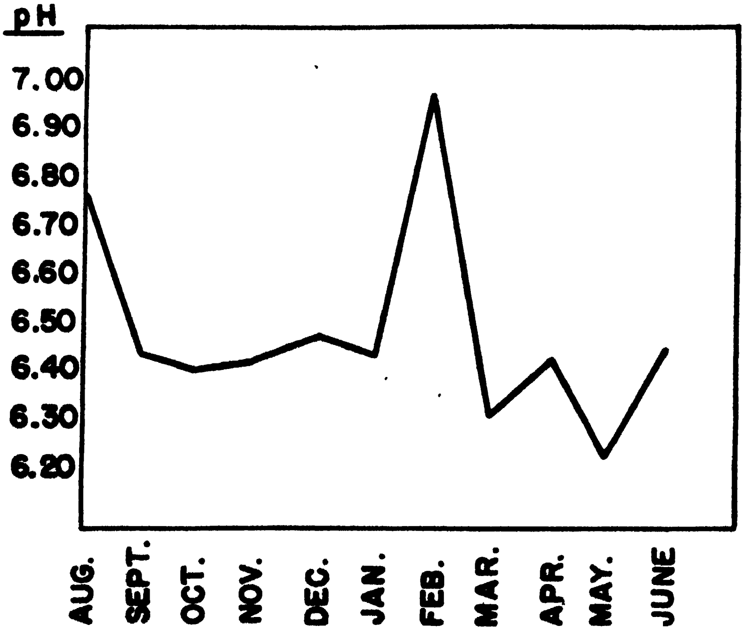

FIG. 1. Seasonal trend in pH based on differences between samples from same bulls.

Anderson (1, p. 137) previously reported the significant differences of pH between individual bulls. The work reported here substantiated his findings. Phillips *et al.* (6) have shown significant correlations of several other semen measures to breed.

Anderson (1, p. 137) further observed highly significant monthly differences in semen pH which he attributed to a basic seasonal rhythm in the bull associated with climatic factors.

The monthly fluctuations in the average pH of semen obtained from the same bulls are shown in figure 1. These fluctuations are notably wide, with February showing the highest pH readings and May the lowest.

Swanson and Herman (9) have interpreted the significant monthly variations to be due largely to the adverse effects of winter weather upon the physical well-being and sexual activity of the bull. One might speculate upon what other factors of seasonal variation could influence the pH. Relative humidity, temperature, and changing periods of daylight are definite variations accompanying seasonal cycles. Mercier and Salisbury (5) have reported a significant relationship between light variation and conception rate in artificial breeding.

Seasonal fluctuations in the nutritional level have been held to a minimum as the bulls were not pastured and were fed only the cured roughages. Thus the only change in the feed would be the storage loss of nutrients and vitamin content during the winter and spring periods.

SUMMARY

A study of the pH of 371 semen samples from 66 bulls in five studs was made over a period of 11 months. A highly significant correlation was found between the pH levels and the individual studs. It would appear that the conditions at the stud exerted a marked effect upon the pH level of semen produced. From one stud to another, the pH levels between the first ejaculations and the second were markedly different, further indicating the effect of individual stud management upon semen production.

A positive correlation was observed between the pH readings taken from the semen of the individual bulls within the different breeds. The semen of individual bulls, irrespective of breed, tended to maintain a constant pH level.

A definite seasonal fluctuation could be recognized as there were marked variations throughout the 11 months of study.

The authors wish to express their appreciation to Dr. G. E. Dickerson, formerly Geneticist with the Regional Swine Breeding Laboratory, Ames, Iowa, now associate professor of animal husbandry, Columbia, Mo., for his assistance in setting up the statistical analyses.

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SOME UNIQUE PROPERTIES OF LACTOSE AS A DIETARY CARBOHYDRATE

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A certain inadequacy of lactose as the sole carbohydrate in the diet of rats has been reported by Ershoff and Deuel (7), who also refer to previous reports on this subject. On the other hand, the complete adequacy of lactose as the only carbohydrate in the diet of rats is demonstrated by their growth and survival on a diet of whole milk supplemented by iron, copper, and manganese to prevent the development of dietary anemia.

The adequacy or inadequacy of lactose as a carbohydrate evidently depends upon the amount of lactose ingested or upon the proportion of the total calories of the diet supplied by lactose. The violent reactions of rats to diets containing large amounts of lactose indicate a very great deviation in physiological action of lactose from that of the other usual food carbohydrates.

The laxative action of considerable amounts of lactose in the diets of both mammals and birds is well known. When Ershoff and Deuel (7) fed a fat-free diet containing 73.2 per cent lactose, the animals exhibited a typical severe diarrhea and failed to survive. Mitchell (10) observed the more or less constant occurrence of diarrhea among rats fed on a moderately high (18 per cent) fat diet containing 60 per cent lactose. On this diet, the feces of the rats contained approximately 17 per cent of reducing sugar. The occurrence of diarrhea thus apparently is related to the inability of the rats to utilize completely such a large proportion (50.6 per cent) of total calories of the diet as lactose.

A so-called "flush" or diarrhea commonly is produced in young chickens by the feeding of a special mash containing 25 per cent of dried cheese whey. Ashcraft (1) reported that chickens fed on a ration containing 28 per cent dried cheese whey showed a persistent diarrhea and loss of weight. Beach (3) and Beach and Davis (4) noted that the droppings from chicks fed on a ration containing 20 per cent lactose were of a watery consistency.

Farmers know that the feeding of large amounts of skim milk or whey to pigs produces a flushing, scouring, or purgative action. Spindler *et al.* (14) call attention to the purgative action of large amounts of skim milk in the diet of pigs.

Apparently low concentrations of lactose in the diet of rats cause no obvious symptoms, and the sugar apparently behaves like other food carbohydrates. On the other hand, at high concentrations the action of lactose is

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strikingly different from that of other food carbohydrates. An intermediate range of lactose in the diet may be presumed to exist between these two extremes. Within this intermediate range, either mild or transitory symptoms should be observed.

From the above considerations, it appeared that a study of the influence of lactose in varying daily amounts might well be undertaken as a background for a critical evaluation of existing literature and for the further evaluation of lactose in the diet.

EXPERIMENTAL PROCEDURE

Young rats of the Sprague-Dawley strain weighing 50 to 60 g. were used. The animals were housed in individual cages and unless otherwise specified the diets were fed *ad libitum* over a 12-week period. Feed consumption records were kept and a small amount of water was mixed with the diets to prevent scattering. Food refusals were weighed back at the end of each week so that the results could be studied by weekly intervals. The animals were weighed three times each week and observed for symptoms.

A basal diet consisting of 20 per cent vitamin-free casein, 7 per cent salt mixture (USP XII, salt mix no. 2), 3 per cent butter oil, 2 per cent cod liver oil (USP XII), 1 per cent liver powder concentrate, and 67 per cent corn starch was used. Each 100 g. of this diet was supplemented with the following amounts of accessory factors: 10.0 mg. alpha-tocopherol, 2.0 mg. 2-methyl-naphthoquinone, 3.0 mg. calcium pantothenate, 30.0 mg. niacin, 0.6 mg. riboflavin, 0.6 mg. pyridoxine, 0.6 mg. thiamine, 75.0 mg. choline chloride, 75.0 mg. inositol, and 75.0 mg. para-amino benzoic acid. This diet is capable of supporting growth, gestation, and lactation in rats of the Sprague-Dawley strain, although its performance in these respects is not as good as the stock colony diet composed of natural foodstuffs. When lactose or mixtures of galactose and glucose were incorporated in this diet, they replaced an equivalent weight of starch. Each lot of diet was analyzed for reducing sugar by the Munson-Walker method (2).

The diets fed in the earliest experiment, in which dried cheese whey was used as a source of lactose, contained a high percentage of minerals and the amount was not reduced in the subsequent experiments using USP lactose. Since these studies were completed, Daniel and Harvey (5) have reported that the soluble ash of whey retarded growth and produced diarrhea in rats when whey was used as an ingredient in an infant-feeding formula containing approximately 50 per cent lactose. In order to determine whether the high mineral content of the basal diet could have influenced growth and diarrhea observed in the present work, six young male rats were placed on a 50 per cent lactose diet (7.0 per cent salt mixture) and six litter mates were fed the same diet containing 3.5 per cent salt mixture, the difference being made up by 3.5 per cent cornstarch. No difference in growth, food utilization, or incidence of diarrhea was observed between the two groups over a 6-week period, so apparently the high mineral content of the basal diet has not influenced the results of this study.

RESULTS

Growth and food utilization. The observed growth on the basal diet and on the diet modified to contain 5, 10, 15, 20, 25, 30, 40, and 50 per cent of lactose is shown in table 1. Each group of eight rats consisted of four males and four females. These cumulative 12-week results indicate that up to and including 30 per cent of lactose, these diets produce approximately the same growth and food utilization as does the basal diet. At lactose levels of 40 and 50 per cent, growth is less; at the 50 per cent level, food utilization is less efficient.

TABLE 1

Summarized growth and food utilization data from 12-week experiment

Lactose content of diet	No. of rats	Av. initial weight	Av. final weight	Av. total gain	Av. feed consumption	Feed required for 1-g. gain
%		(g.)	(g.)	(g.)	(g.)	(g.)
Experiment I						
Basal	8	54	277	223	1072	4.81
10	8	55	290	235	1109	4.72
20	8	57	271	214	1072	5.01
30	8	57	275	218	1052	4.83
40	8	56	254	198	1005	5.08
50	8	58	235	177	973	5.50
Experiment II						
Basal	8	51	279	228	1114	4.89
5	8	51	282	231	1126	4.87
10	8	51	293	242	1170	4.83
15	8	49	282	233	1142	4.90
20	8	49	280	231	1140	4.94
25	8	50	272	222	1111	5.00

Relation of appearance of diarrhea to lactose intake. Significant as the above cumulative results may be, the reactions and symptoms of the animals early in the experiment must be considered. When young rats were placed on diets containing the higher levels of lactose, feed intake was reduced and they suffered from diarrhea. Since the symptoms were most prevalent during the first 4 weeks of the experiment, the approximate lactose consumption per day per kilogram of body weight has been calculated for each of the first 4 weeks and is shown in table 2. The body weights used for these calculations were averages of the initial and final weight for each weekly interval, and the system of weighing back food refusals at the end of each week gave the lactose consumption for the week. The appearance and severity of diarrhea during each week also are shown. Diarrhea disappeared after approximately 3 weeks in rats receiving diets containing 30 per cent lactose, but these animals were growing at a relatively rapid rate and weighed in the neighborhood of 170 g. at the end of 4 weeks. However, when the data are calculated to the basis of grams of lactose consumed per kilogram of body weight, the animals apparently were able to use larger amounts of lactose without having diarrhea after being on their respective lactose diets for a few days.

During the first week there was some evidence of diarrhea on the 20 per cent lactose diet at a lactose intake of 22.2 g. per kg. of rat per day. On diets containing more lactose and where lactose intake was greater, the animals suffered from severe diarrhea during the first week. During the second week, diarrhea was not evident until the rats consumed 27.5 g. lactose per kg. body weight per day on the 25 per cent lactose diet. During the third week there was no severe diarrhea, and during the fourth week it was observed only in rats on the 40 and 50 per cent lactose diets.

TABLE 2

Approximate amount of lactose consumed per day
(Grams per kilogram body weight)

Lactose content of diet	1st week	2nd week	3rd week	4th week
(%)	(*g.*)	(*g.*)	(*g.*)	(*g.*)
5	6.1	6.1	5.3	4.3
10	12.3	12.3	10.4	8.9
15	18.0	18.7	15.6	14.4
20	22.2*	24.4	21.0	19.8
25	28.5†	27.5‡	27.8	25.2
30§	27.5†	28.2‡	29.4*	25.6
40§	36.1†	36.4†	38.4‡	36.0*
50§	42.7†	47.7†	44.9‡	42.5*

* Some evidence of diarrhea.
† Severe diarrhea.
‡ Moderate diarrhea.
§ These results are from experiment I; all others are from experiment II.

In addition to the data shown in tables 1 and 2, the following additional observations should be pointed out. Rats on diets containing 30, 40, and 50 per cent lactose developed a "pot-bellied" appearance, which persisted after the diarrhea cleared up but which disappeared before the end of the 12-week test period. Some of the animals on diets containing 40 and 50 per cent of lactose had rough coats during the first 3 or 4 weeks of the test.

Adaptation to lactose. Since the capacity of the rats to utilize rations containing moderate amounts of lactose without having diarrhea apparently increased with length of time on the rations, this aspect was studied further. Forty-eight young male rats were divided into six groups of eight rats each. Table 3 gives the feeding schedule for each group during the 6-week test

TABLE 3

Feeding schedule of diets followed in studying adaptation to lactose

Group	No. of rats	1st week	2nd week	3rd week	4th week	5th week	6th week
1	8	10% lactose	20% lactose	30% lactose	40% lactose	50% lactose	60% lactose
2	8	Basal	20% lactose	....	..	..	.
3	8	"	Basal	30% lactose	. .	. .	.....
4	8	"	"	Basal	40% lactose	..	.
5	8	"	"	"	Basal	50% lactose	.
6	8	"	"	"	"	Basal	60% lactose

period. The rats of group 1 were continued throughout the experiment, their lactose intake being stepped up each week through the feeding of diets containing a larger percentage of lactose. The rats in the other groups were discarded following the 1-week test period on a lactose diet.

Beginning in the second week, all rats were weighed daily and feed intake regulated in such a way as to keep the rats on the basal diet growing at the same rate as the two groups on the lactose diet. An effort was made to keep the two groups on the lactose diet growing at the same rate. As the percentage of lactose increased, it became more difficult to keep the rats just placed on the diet growing as rapidly as those in group 1, where the lactose intake had been increased gradually. Clean papers were placed under the rats each day except Sundays, and these were observed the following morning for evidence of diarrhea.

Table 4 gives the average weights and feed consumption on each diet,

TABLE 4

Adaptation to lactose—summarized growth and food utilization data

Lactose content of diet*	Week of test	Group no.	Initial weight	Weight at end of 7 days	Gain	Feed consumed	Feed required for 1-g. gain
(%)			(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)
20	2nd	1	61.4	87.5	26.1	50.7	1.94
20	"	2	63.0	86.6	23.6	49.2	2.08
30	3rd	1	87.5	115.0	27.5	66.1	2.40
30	"	3	86.5	115.6	29.1	74.5	2.56
40	4th	1	115.0	139.3	24.3	80.4	3.31
40	"	4	116.1	136.9	20.8	80.6	3.88
50	5th	1	139.3	156.9	17.6	74.9	4.26
50	"	5	142.1	151.5	9.4	72.2	7.68
60	6th	1	156.9	159.1	2.2	64.7	29.41
60	"	6	155.8	151.9	−3.9	55.1	

* Previous dietary history of each group is shown in table 3.

both for the rats of group 1, in which lactose intake had been increased gradually, and for those which were transferred from the basal diet to the lactose diet. Figure 1 shows the incidence of diarrhea in each group. Since there was no diarrhea at the 10 per cent level, and gain and food utilization were equal to the groups on basal diet, the results of the first week are not shown in the table or the figure.

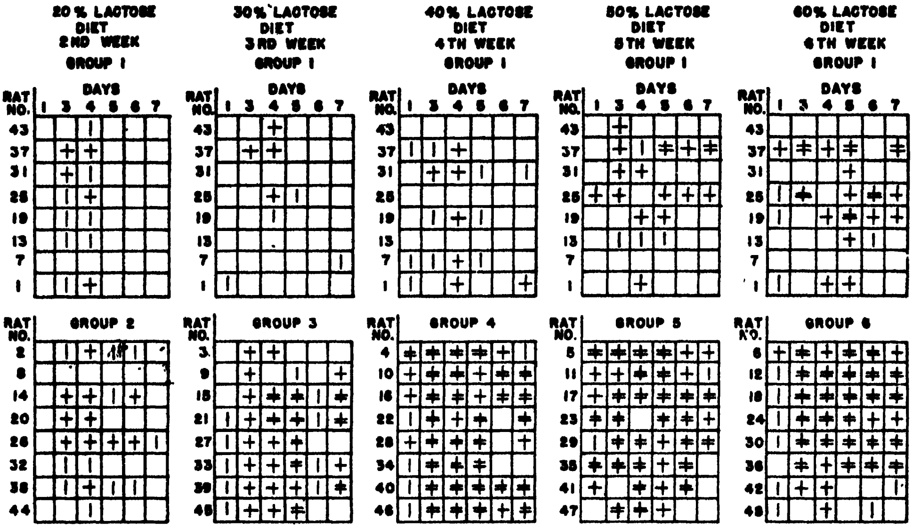

FIG. 1. Occurrence and degree of diarrhea.
| — Some evidence of diarrhea, *i.e.*, some unformed feces.
+ — Moderate diarrhea, *i.e.*, considerable unformed feces.
$\ddagger$ — Severe diarrhea, *i.e.*, large amounts of watery feces.

On the 20 per cent lactose diet, the difference in the incidence of diarrhea between the two groups was not great, and in no case was it severe. However, diarrhea cleared up entirely after 4 days in the group which previously had been fed the 10 per cent lactose diet, and it continued beyond this period in four of the eight rats in the other group. The small difference in grams of food required for a gram gain between the two groups was not statistically significant when the data were treated by Student's "*t*" test for paired differences ($P > 0.2$). The lactose intake for the rats previously on the 10 per cent lactose diet was 19.4 g. per kg. per day, whereas it was 18.8 g. per kg. per day for the other group. Both of these figures for lactose intake fall between the values where diarrhea was absent and where it first occurred during the first week, as shown in table 2.

On the 30 per cent lactose diet diarrhea was much more prevalent and much more severe in the group transferred from the basal diet. The lactose intake was 28.5 g. per kg. per day in the group in which lactose intake had been increased gradually, and 31.4 g. per kg. per day in the group transferred from the basal diet. These values fall in the range that produces severe diarrhea during the first week, as shown in table 2. As a matter of fact, all figures for lactose intake higher than 30 per cent fall within the range that produces severe diarrhea during the first week on lactose diets. The difference in grams of feed required to produce a gram gain in weight between the two groups on 30 per cent lactose diet is not significant ($P > 0.4$). The difference in occurrence and severity of diarrhea between the two groups on the diet containing 40 per cent lactose is very striking, but here again the difference between the grams of feed required to produce a gram gain in weight is not statistically significant ($P > 0.1$).

Diarrhea was much more prevalent and more severe in the group transferred from basal diet to the diet containing 50 per cent lactose than it was in the group in which lactose intake had been increased gradually. One rat which was transferred from the basal diet to the diet containing 50 per cent lactose gained nothing and another lost 1 g. over the 7-day period. All eight rats previously fed diets containing lactose gained during this period.

Six of the eight rats just placed on the diet containing 60 per cent lactose lost weight, and three of the eight for which lactose intake had been increased also lost weight. Here again diarrhea was less severe in the rats which previously had been fed lactose.

These results confirm the observation that the ability of the rat to utilize diets containing lactose without suffering from diarrhea increases with length of time on such diets. In the present experiment, such variables as age and weight of the animals being compared have been eliminated. However, these differences in the incidence and severity of diarrhea were not in every case associated with significant differences in utilization of the total diet as measured by grams of food required to produce a 1-g. gain, although all average numerical values for food utilization favor rats previously on lactose diets.

Certain individual variations among the rats with regard to incidence and severity of diarrhea are apparent from figure 1 and cannot be explained on the basis of food intake for the period. Rat no. 37, of the group in which lactose intake gradually had been increased, ate 75 g. of 50 per cent lactose diet (37.5 g. lactose) during the week and exhibited diarrhea on 5 of the 6 days on which observations were made. On the other hand, rat no. 7 of this same group never had diarrhea even though it ate 80 g. of diet (40 g. lactose) during the same period.

Influence of lactose linkage on utilization. This experiment was carried out to determine if the lactose linkage was responsible for any of the physiological disturbances associated with lactose feeding. Each of the following diets was fed to a group of eight young male rats: (a) 15 per cent lactose, (b) 7.5 per cent galactose plus 7.5 per cent glucose, (c) 30 per cent lactose,

(d) 15 per cent galactose plus 15 per cent glucose, (e) 50 per cent lactose, and (f) 25 per cent galactose plus 25 per cent glucose. These levels of lactose and the corresponding levels of the mixture of equal parts galactose and glucose were chosen because when 15 per cent lactose was fed, no symptoms were found to occur. With 30 per cent lactose, the symptoms were transitory, and with 50 per cent lactose, symptoms were persistent and more severe.

The results of the 12-week growth test are shown in table 5. No symptoms of intestinal disturbances occurred in the rats receiving the mixture of galactose and glucose at any of the three levels, whereas the rats receiving lactose reacted in the usual manner previously described in this report. One rat on the diet containing 25 per cent galactose plus 25 per cent glucose developed a mature cataract by the end of the 12-week test.

TABLE 5

Summarized data comparing lactose with glucose and galactose mixture

Diet	No. of rats	Av. initial weight	Av. weight at end of 12 weeks	Av. total gain	Av. food consumption	Feed required for 1-g. gain	Symptoms
		(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	
7.5% glucose + 7.5% galactose	8	55	330	275	1197	4.35	None
15% lactose	8	53	332	279	1198	4.29	None
15% glucose + 15% galactose ...	8	52	313	261	1346	5.16	None
30% lactose	8	51	292	241	1032	4.28	*
25% glucose + 25% galactose	8	57	288	231	1305	5.65	None
50% lactose . . .	8	53	219	166	802	4.83	†

* Severe diarrhea first and second week, some diarrhea third week, none thereafter.

† Severe diarrhea first and second week, moderate diarrhea third and fourth week, some diarrhea fifth week.

There was no difference in rate of growth, total gain, or food utilization between the rats on the 15 per cent lactose diet and those on the diet containing 7.5 per cent glucose plus 7.5 per cent galactose. The rats on the 30 per cent lactose diet grew more slowly early in the test period and gained slightly less than those on the diet containing 15 per cent glucose plus 15 per cent galactose. However, food utilization was more efficient on the 30 per cent lactose diet than on the 15 per cent glucose plus 15 per cent galactose diet. Growth was considerably less on the 50 per cent lactose diet than on the 25 per cent glucose plus 25 per cent galactose diet, but food utilization was more efficient on the 50 per cent lactose diet.

Growth curves drawn from the average weights of each group are shown in figure 2. These growth curves are included since the growth curves for the lactose rats are typical for rats on these diets and they demonstrate the retarded rate of growth on diets containing large amounts of lactose during the early part of the test period. In the previous experiment an average gain of 10 g. per rat was obtained during the first week on the 50 per cent lactose diet, whereas in the present experiment there was an average loss of 1 g.

These results indicate that the inability of the rat to hydrolyze large amounts of lactose is responsible for the diarrhea and related symptoms of distended abdomens and slower rate of growth associated with high levels of lactose in the diet.

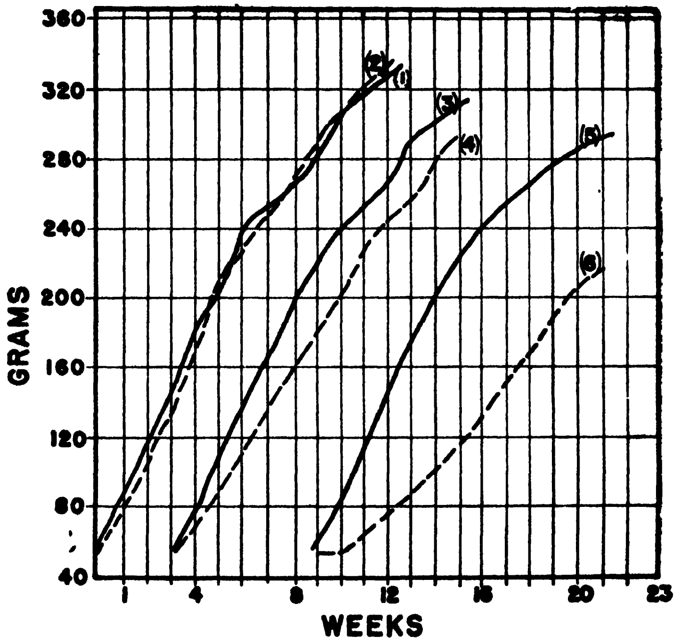

FIG. 2. Growth curves of rats fed diets containing lactose and equivalent amounts of glucose and galactose.

(1) — 7.5% glucose plus 7.5% galactose.
(2) — 15% lactose.
(3) — 15% glucose plus 15% galactose.
(4) — 30% lactose.
(5) — 25% glucose plus 25% galactose.
(6) — 50% lactose.

Lactose in natural milk products. Dried cheese whey was incorporated as a source of lactose in the basal diet, adjustments being made for the amount of protein and minerals present in the whey and covering the same range reported for USP lactose. Results with dried whey were comparable to those with lactose at each level both with regard to rate of growth, food utilization, and typical symptoms on the moderate and high levels of lactose as shown in tables 1 and 2.

When nonfat dry milk solids supplemented with a daily dose of 0.1 ml. USP cod-liver oil and iron, copper, and manganese were fed as the sole article of diet to young growing rats, their response followed the usual pattern obtained on high lactose diets. The particular lot of nonfat dry milk solids fed contained 54.6 per cent lactose.

When dried whole milk containing 27.5 per cent fat and 37.5 per cent lactose was supplemented as for nonfat dry milk solids and fed as the sole article of diet, rate of growth was rapid and the animals had only a slight degree of diarrhea during the first 2 or 3 days on the diet. Lactose intake was approximately 34.3 g. per kg. per day during the first week of the test, and approximately 33.2 g. per kg. per day during the second week. On the low-fat diet, such a large intake of lactose would have produced severe symptoms during this period. It is unlikely that fat affects lactose hydrolysis *per se,* but the effect of fat on lactose and its relation to diarrhea should be investigated further.

DISCUSSION

Mitchell (11) has reported that growth of rats was poor on diets containing 60 per cent or 45 per cent of lactose, but that it was practically normal on diets containing 30 per cent of lactose or less. She used a diet containing 18 per cent fat and lactose at levels of 5, 15, 30, 45, and 60 per cent. The authors have found that rats weigh as much at the end of 12 weeks on a 30 per cent lactose diet as they do on the basal diet containing cornstarch, but they grow more slowly early in the test. Judging from results on dried whole milk which contains 27.5 per cent fat, the higher percentage of fat in Mitchell's diet may have resulted in either lower lactose intake or better utilization of the ingested lactose than was the case on the diet used in these experiments, which contained only 5 per cent fat.

Results indicate that through continued daily intake, the rat can increase its capacity to utilize lactose without suffering from diarrhea. Nevertheless, there appears to be a limit to the amount of lactose the rat can utilize efficiently as a source of carbohydrate; there appears to be individual as well as strain differences in this respect. In addition to continued intestinal disturbances at the higher levels of lactose intake, development of cataract has been described by Mitchell and Dodge (12). Whittier *et al.* (15) have observed that rats have diarrhea on diets containing 45 per cent or 63.5 per cent lactose and 7.5 per cent fat for a period of 3 weeks, but that they then recover and make good gains. The present authors have found that diarrhea persists on diets containing 5 per cent fat and 40 per cent and 50 per cent lactose, although it was intermittent and less severe during the latter part of the 12-week test period.

Mitchell *et al.* (9) have pointed out the impairment in the digestion of organic nutrients by lactose. Present results indicate that the lactose linkage is a primary factor in producing any ill effects from lactose feeding within the ranges reported herein and in the type of diet used.

It has been shown by Schantz *et al.* (13), Geyer *et al.* (8), and Zialcita and Mitchell (16) that galactose is excreted in the urine of rats fed either galactose or lactose. This failure fully to utilize galactose at the higher levels probably accounts for the slower rate of growth and the larger amount of food required for a gram of gain in weight as the amount of galactose is increased from 7.5 to 25 per cent of the total ration. However, the pronounced difference in growth between a mixture of galactose and glucose and an equivalent amount of lactose at the 30 per cent and 50 per cent levels indicates that failure to hydrolyze the lactose linkage exerts a greater influence than the loss of galactose.

Ershoff (6) fed mixtures of galactose and glucose or lactose to rats as the sole article of diet. On lactose the rats survived an average of 6.9 days, whereas they survived an average of 31.2 days on a mixture of 50 per cent glucose and 50 per cent galactose. He concluded that the rats were not able to hydrolyze lactose in adequate amounts. The authors' results indicate that such is the case even when large amounts of lactose are ingested as part of a relatively adequate diet.

CONCLUSIONS

1. When purified low-fat diets containing 5, 10, and 15 per cent lactose were fed to young growing rats, growth and food utilization were normal and no symptoms attributable to lactose were observed.
2. When the diet contained 20, 25, and 30 per cent lactose, transitory symptoms of lactose feeding (diarrhea and/or "pot bellies") appeared. However, growth and food utilization were almost the same as on a basal ration containing no lactose.
3. When the lactose content of the diet was increased to 40 and 50 per cent lactose, growth was less; at the 50 per cent level, food utilization was less efficient. Symptoms of lactose feeding were more severe and persistent.
4. The capacity of the rat to utilize lactose without suffering from diarrhea can be increased through a continued daily intake of rations containing lactose.
5. By feeding rations containing a mixture of equal parts of glucose and galactose at levels equivalent to 15, 30, and 50 per cent lactose, it was found that the lactose linkage was responsible for symptoms of lactose feeding observed on rations containing higher percentages of lactose.
6. At the 30 per cent level, growth was slightly less, and at the 50 per cent level, growth was considerably less on lactose than on the mixture of

equal parts of glucose and galactose. However, at both levels, food utilization was much more efficient on the lactose diets.

7. In planning nutrition studies wherein lactose is a component of the ration, account must be taken of the limits within which the rat can utilize this sugar adequately as well as the other ingredients of the diet which may influence lactose intake and utilization.

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A COMPARISON OF TOTAL MILK AND FAT PRODUCTION AND PER CENT FAT OF A 26- AND 24-HOUR TEST DAY[1,2,3]

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All dairymen know that cows produce more milk after a long than a short interval. In the standard-type Dairy Herd Improvement Association, a night milking of one day and the morning milking of the successive day are weighed and tested for fat once a month. The lactation production of a cow is computed from these monthly milk weights and fat tests. If the milking schedule is altered, intentionally or unintentionally, at the morning milking preceding the first weighing and sampling or the final milking of the test period, or both, the milk and fat production will be affected.

A questionnaire prepared by the authors was sent to D.H.I.A. supervisors in Wisconsin. Twenty-nine questionnaires were answered. Nine of the supervisors indicated that from 5 to 30 per cent of their members altered the milking schedule in anticipation of the arrival of the supervisor, so that the test day was lengthened from 0.5 to 2 hours. Consequently, experiments were designed to determine the difference in milk and fat production of cows milked at two 13-hour and two 12-hour intervals to provide a 26- and a 24-hour test day.

EXPERIMENTAL PROCEDURE

Experiment I. Four Holstein-Friesian cows were selected for the study to form two pairs with comparable levels of production. The experiment consisted of a one-day preliminary period and 4 test days, with a transition period of one day between test days. One-half of the udder was milked at 13-hour intervals and the other half at 12-hour intervals on each day. The halves of the udder were alternated between the two milking intervals from one test day to the next in order to form an experiment of double switchback design. In this experiment one-half of the udder served as a control for the other half. Ludwick *et al.* (2) used a similar design in comparing production of 2- and 3-times-a-day milking. Table 1 illustrates the milking schedule. Ten I.U.'s of oxytocin were injected intravenously immediately before each milking to insure a complete "let-down" of milk at

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[1] These data were taken from a thesis presented to the Graduate School of the University of Wisconsin by T. H. Blosser in partial fulfillment of the requirements for the degree of Master of Science.

[2] The especially designed milking machine used in these studies was made available through the courtesy of the Ben H. Anderson Mfg. Co., Madison, Wis.

[3] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

each milking. A milking machine designed to milk each quarter into a separate container was used.

Experiment II. Two groups of cows representing four breeds, with 11 cows in each group, were used in this experiment. The cows is these groups were paired with regard to level of production, breed, and stage of lactation. Experiment II differed from experiment I in that one group of cows served as controls for the other group, and the entire udder was milked at each milking. The experiments were alike in that a double switchback design was used in both. The milking schedule for experiment II may be had by substituting group 1 for "left half of udder" and group 2 for "right half of udder" in the respective columns of table 1.

TABLE 1

Milking schedule for experiment I

Left half of udder				Right half of udder			
Time of milking	Interval between milkings	Length of test day	No. of test day	Time of milking	Interval between milkings	Length of test day	No. of test day
	(*hr.*)	(*hr.*)			(*hr.*)	(*hr.*)	
a.m. 5: 30				a.m. 5: 30			
p.m. 5: 30				p.m. 5: 30			
a.m. 5: 30				a.m. 4: 30			
	12				13		
p.m. 5: 30				p.m. 5: 30			
	12				13		
a.m. 5: 30		24	I	a.m. 6: 30		26	I
p.m. 5: 30				p.m. 5: 30			
a.m. 4: 30				a.m. 5: 30			
	13				12		
p.m. 5: 30				p.m. 5: 30			
	13				12		
a.m. 6: 30		26	II	a.m. 5: 30		24	II
p.m. 5: 30				p.m. 5: 30			
a.m. 5: 30				a.m. 4: 30			
	12				13		
p.m. 5: 30				p.m. 5: 30			
	12				13		
a.m. 5: 30		24	III	a.m. 6: 30		26	III
p.m. 5: 30				p.m. 5: 30			
a.m. 4: 30				a.m. 5: 30			
	13				12		
p.m. 5: 30				p.m. 5: 30			
	13				12		
a.m. 6: 30		26	IV	a.m. 5: 30		24	IV

In both experiments, the night milking of one day and the morning milking of the consecutive day constituted a test day. The milk produced at each milking was weighed and a sample was taken for fat analysis. Fat analyses were made on each milking by the Babcock method. The data of these experiments were statistically analyzed by the method developed by Brandt (1) for reversal or switchback trials.

TABLE 2

Milk and fat production and per cent fat for each half of the udder in experiment I

	Production											
Test day	I			II			III			IV		
	Right half of udder											
Length of test day	26 hr.			24 hr.			26 hr.			24 hr.		
No. of cow	Milk (*lb.*)	Fat (%)	Fat (*lb.*)	Milk (*lb.*)	Fat (%)	Fat (*lb.*)	Milk (*lb.*)	Fat (%)	Fat (*lb.*)	Milk (*lb.*)	Fat (%)	Fat (*lb.*)
239	17.8	3.65	0.65	18.5	3.75	0.69	19.4	3.67	0.71	18.3	3.75	0.69
282	30.7	5.40	1.66	29.3	5.09	1.49	30.7	3.93	1.21	34.0	4.70	1.60
359	21.4	3.50	0.74	19.0	3.21	0.61	22.0	3.25	0.72	20.0	3.36	0.67
258	33.6	3.25	1.09	30.6	3.05	0.93	31.9	3.01	0.96	31.0	2.99	0.93
	Left half of udder											
Length of test day	24 hr.			26 hr.			24 hr.			26 hr.		
No. of cow												
239	18.1	3.95	0.72	20.2	3.60	0.72	19.0	3.90	0.74	21.0	3.45	0.72
282	28.5	4.92	1.40	32.7	4.71	1.54	30.8	4.53	1.39	35.9	3.43	1.23
359	23.5	3.56	0.84	25.7	3.05	0.78	24.7	3.45	0.85	25.6	3.10	0.79
258	31.9	3.40	1.09	32.8	3.25	1.07	28.3	3.20	0.90	33.6	3.00	1.01

TABLE 4

Daily milk and fat production and per cent fat of individual cows in experiment II

No. of cow	Milk (lb.)	Fat (%)	Fat (lb.)	Milk (lb.)	Fat (%)	Fat (lb.)	Milk (lb.)	Fat (%)	Fat (lb.)	Milk (lb.)	Fat (%)	Fat (lb.)
Group I												
	Test day and length											
	I—24 hr.			II—26 hr.			III—24 hr.			IV—26 hr.		
211	53.2	3.14	1.67	58.5	3.11	1.82	54.8	3.76	2.06	57.8	3.20	1.85
891	39.6	3.66	1.45	47.3	3.91	1.85	40.5	3.46	1.40	44.9	3.70	1.66
703	34.1	4.31	1.47	35.7	4.31	1.54	34.4	5.09	1.75	39.5	4.81	1.90
F552	30.5	3.70	1.13	36.1	3.85	1.39	29.3	3.82	1.12	35.3	3.91	1.38
B63	29.4	4.69	1.38	32.0	4.59	1.47	31.4	5.10	1.60	32.6	4.66	1.52
696	29.8	6.04	1.80	30.9	5.31	1.64	29.6	5.54	1.64	33.0	5.55	1.83
705	28.0	5.64	1.58	30.8	4.87	1.50	26.2	6.18	1.62	26.5	5.40	1.43
B57	23.2	5.65	1.31	24.0	5.17	1.24	23.7	5.91	1.40	24.7	5.51	1.36
259	23.2	4.09	0.95	23.8	3.74	0.89	21.3	3.76	0.80	25.1	3.90	0.98
292	17.3	3.93	0.68	18.7	3.58	0.67	16.3	3.87	0.63	19.2	3.91	0.75
B61	16.7	5.99	1.00	18.3	5.74	1.05	16.1	6.70	1.08	15.9	6.10	0.97
Group II												
	Test day and length											
	I—26 hr.			II—24 hr.			III—26 hr.			IV—24 hr.		
874	51.3	3.61	1.85	49.4	3.44	1.70	48.5	3.55	1.72	41.5	3.64	1.51
555	41.8	5.14	2.15	38.8	5.26	2.04	39.6	4.37	1.73	38.8	5.72	2.22
579	39.8	3.82	1.52	37.2	4.27	1.59	38.4	3.65	1.40	36.4	4.15	1.51
575	34.8	5.29	1.84	27.4	4.45	1.22	31.1	4.15	1.29	28.2	4.82	1.36
541	32.6	4.51	1.47	28.6	4.69	1.34	32.6	4.36	1.42	30.6	4.54	1.39
1723	28.4	3.35	0.95	24.1	4.02	0.97	26.2	3.85	1.01	25.9	3.24	0.84
578	22.6	5.18	1.17	20.7	5.17	1.07	21.7	5.02	1.09	20.6	5.49	1.13
1438	20.7	3.96	0.82	21.2	3.68	0.78	21.6	3.66	0.79	20.4	3.77	0.77
1503	19.1	3.09	0.59	17.9	3.63	0.65	19.4	3.40	0.66	18.3	3.39	0.62
256	18.0	2.78	0.50	17.4	3.91	0.68	17.6	3.69	0.65	18.6	3.71	0.69
588	11.5	4.17	0.48	12.9	5.04	0.65	13.3	4.66	0.62	13.0	4.92	0.64

TABLE 3

Average test-day production of milk, fat, and per cent fat of one-half of the udder for all experimental periods of experiment I

	Milk	Fat	Fat
	(*lb.*)	(*lb.*)	(%)
26-hr. test day	27.2	0.98	3.59
24-hr. test day	25.3	0.97	3.83
Difference	1.9	0.01	− 0.24
% increase	7.50	1.03	− 6.26

RESULTS

The results of Experiment I are presented in table 2 and summarized in table 3. With but four exceptions in 32 comparisons, the half of the udder milked at 13-hour intervals to provide 26-hour test days produced more than the same half of the udder milked at 12-hour intervals for 24-hour test days. It is noted in table 3 that 7.5 per cent more milk was produced in a 26- than in a 24-hour test day. On statistical analysis this increase in milk production proved to be highly significant.

There was no consistent trend in total fat production. Greater fat production resulted in 16 out of 32 comparisons for the 26-hour test days, 14 out of 32 for the 24-hour test days, and in two comparisons the production was equal. Differences in fat production for the 26- and 24-hour test days were not statistically significant.

The results of Experiment II are presented in table 4 and summarized in table 5. Milk production was greater for the 26-hour test days in 79 of the 88 comparisons. Statistical analysis showed this increase in production, 8.5 per cent, to be highly significant.

The per cent fat was higher for the 24-hour test days in 63 of 88 comparisons. The difference was found to be statistically significant.

Total fat production was quite variable. More fat was produced in the 26-hour test days in only 48 of the 88 comparisons. The differences in fat production was not statistically significant.

DISCUSSION

Both experiments of this study used a double switchback design. However, in the first experiment one-half of the udder was used as a control for

TABLE 5

Average test-day production of milk, fat, and per cent fat of all cows for all experimental periods of experiment II

	Milk	Fat	Fat
	(*lb.*)	(*lb.*)	(%)
26-hr. test day	30.5	1.28	4.20
24-hr. test day	28.1	1.25	4.44
Difference	2.4	0.03	− 0.24
% increase	8.47	2.40	− 5.40

the other half, thus providing a within-a-cow control. In the second experiment one group of cows acted as controls for the other. The results obtained by these two methods of experimentation checked one another closely.

SUMMARY AND CONCLUSIONS

1. Differences in total milk production for a 26- and 24-hour test day are statistically significant, with a higher production of milk for the 26-hour test day.
2. Differences in per cent fat for a 26- and 24-hour test day are statistically significant, with a lower per cent of fat for the 26-hour test day.
3. Differences in total fat production for a 26- and 24-hour test day are not statistically significant.
4. Dairymen enrolled in D.H.I.A. who anticipate a test day and alter their milking time in order to obtain longer intervals between milkings probably will increase total milk production, decrease the per cent of fat of the milk, and not appreciably influence total fat production.

The authors wish to express their thanks to Dr. J. E. Torrie and Mrs. Dorothy Brill for the statistical analyses of these data.

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REPEATABILITY OF HEAT TOLERANCE OBSERVATIONS

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Interest in repeatability of observational data as it relates to heat tolerance in dairy cattle is primarily two-fold. Data on repeatability furnish information as to which observations are the most reliable and furnish a method of determining the number of observations necessary to arrive at a reasonably accurate estimate of the relative ability of animals to withstand heat. Also, as explained by Lush (1), a high percentage of the repeatability between observations of the same animal is due to inheritance. Because of this relationship, an estimate of heritability often can be made by taking approximately two-thirds to four-fifths of the repeatability figure as that for heritability. Exceptions to this rule occur, however.

In previous studies at this Station, the authors (2, 3, 4, 5) have reported observations on cows made immediately after entering the milking barns, at 3:30 p.m. An analysis of the data thus secured permitted estimates to be made of heritability and repeatability of body temperature and respiration rates as characteristics for appraising the heat tolerance of Jersey and Holstein milking cows. The body temperature estimates for 1944 and 1945, based on data gathered on 8 warm days for each year, were 0.152 and 0.385 for repeatability and 0.151 and 0.309 for heritability, respectively.[2] Thus, the heritable portion of the variance was only slightly less than that found for repeatability between records of the same cow after removing differences between herds and breeds. Respiration rates analyzed on this same basis gave repeatability figures of 0.42 and 0.48 for the 2 years and estimates of heritability of 0.76 and 0.84. In this case the estimates of heritability exceeded those for repeatability. This was explained as sampling error and was well within the normal fiducial limits at the 5 per cent level of probability.

The present study was undertaken primarily in order to determine whether heat tolerance observations as determined after cows enter the milking barn were comparable to and as repeatable as observations taken on the

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[1] Dairy husbandman and assistant in dairy husbandry, respectively. Acknowledgment is made to Cecil Branton, assistant dairy husbandman, for suggested changes made in the final manuscript.

[2] The computations (8) of repeatability of single records of the same cow and heritability of single records of cows involved the segregation of specific sources of variance and use of the principles involved in intra-class correlations. The first is a measure of how well records of the same cow repeat themselves on various test periods and the second is a measure of what portion of the variance between single records of different cows is hereditary.

same cows while they were in the shade in the pasture and just prior to going to the milking barn. A second portion of the study was concerned with determining how many observations were necessary in order to attain a high degree of accuracy in selecting cattle for a high tolerance to heat.

EXPERIMENTAL PROCEDURE

Twenty milking Holstein cows that also were utilized in a pasture grazing experiment (7) were used in this experiment. At 2:45 p.m. and just prior to going into the milking barn (at 3:30 p.m.), while cows were still in the shade, respiration counts from flank movements of each cow were made by two different workers. Whenever these counts differed by more than 2 for 0.5-minute periods, a third count was made. Approximately 5 to 10 minutes after the cows had entered the milking barn respiration rates again were determined by using the same procedure as was used while cows were in the pasture. Body temperatures of the cows were secured by inserting veterinary thermometers approximately 2.5 inches into the rectum. It required about 30 minutes to complete observations in pasture and 40 minutes for those in the barn. Variation in the order of observing cows was made from day to day in order to equalize any influence causing changes from hour to hour.

Data from observations made during 14 days were secured in the summer of 1946 on selected days between July 17 and September 6. In general, only the clear warm days were used. Air temperatures at 2:45 p.m. for these days varied from 78 to 92° F. and averaged 88° F. Relative humidity varied from 44 to 82 per cent and averaged 60 per cent.

RESULTS

Respiration—mean values and ratings. Mean respiration rates (table 1) for the 14-day period that cows were under observation averaged 78.5 at 2:45 while cows were in shade within pasture and 80.4 after cows entered the milking barn at 3:30 p.m. Observations made on 13 of the 20 cows averaged higher for observation within the barn. On the other hand, observations made on the seven remaining cows averaged higher when made in the pasture. It would appear from these results that the walk from the pasture shade into the milking barn tended to accelerate the respiration rates of the majority of the cows. This occurred even though a period of approximately 5 to 10 minutes was allowed between the time that cows entered milking barn and the time that the first observations were made. To complete all observations required approximately 40 minutes; thus the average time that a cow stayed in the barn prior to observation was around 25 minutes. The order of observing cows was changed from day to day, thus making it as nearly uniform as possible for all cows.

Some small differences were noted among the relative ratings (table 1) based on pasture observations and those on barn observations. For example,

TABLE 1

Comparative mean values and rank of respiration rates of 20 cows (taken in pasture and in barn during mid-afternoon)

Cow no.	14-day mean respiration rates		Difference	Relative rank	
	At 2: 45 p.m. in pasture	At 3: 30 p.m. in barn		In pasture	In barn
	(*per min.*)	(*per min.*)			
1	67.7	69.1	+ 1.4	17	17
2	64.0	69.9	+ 5.9	19	16
3	81.2	80.4	− 0.8	9	11
4	86.7	94.4	+ 7.7	3 (tie)	2
5	83.1	79.9	− 3.2	7	12
11	65.5	65.9	+ 0.4	18	19
12	77.5	82.4	+ 4.9	12	8
13	93.2	86.7	− 6.5	2	5
14	85.3	82.4	− 2.9	6	9
15	99.4	103.0	+ 3.6	1	1
21	77.0	79.6	+ 2.6	13 (tie)	13
22	80.9	85.6	+ 4.7	10	6
23	77.0	83.6	+ 6.6	13 (tie)	7
24	86.7	94.0	+ 7.3	3 (tie)	3
25	54.6	63.2	+ 8.6	20	20
31	78.9	80.6	+ 1.7	11	10
32	75.2	74.0	− 1.2	15	15
34	85.9	91.8	+ 5.9	5	4
35	68.4	65.9	− 2.6	16	18
36	82.0	76.1	− 5.9	8	14
Av.	78.5	80.4	+ 1.9		

the ten cows rating highest on pasture observations include three cows not among the top ten when rated on barn observations. Likewise, the high ten from the barn observations include two cows not found among the high ten based on pasture observations. In spite of these differences, the ratings are much alike, with the same cow rating top (1st) and the same one bottom (20th) in both ratings.

Respiration—repeatability. An analysis of the respiration data for repeatability (table 2) (8) results in estimates for the 14 days studied. Only small differences in repeatability between the two observation periods were

TABLE 2

An analysis of variance for respiration observations taken in pasture and in barn

Source of variance	Degrees of freedom	Mean squares		Composition of mean square
		Pasture data	Barn data	
Between cows	19	1562.74	1565.00	$E + cC$
Within cows	260	171.95	127.55	E

c = number of observations per cow, or 14.

C = variance due to cow = $(E + cC) - E = 99.34$ (for pasture data), 102.68 (for barn data).

Repeatability $= \frac{C}{E + C} = 0.366$ (for pasture data), 0.446 (for barn data).

found. The pasture observation data indicated a repeatability of 0.366, while that from the barn was 0.446. The small difference found would tend to favor slightly observations made in the barn, as has been the routine procedure in previously reported Louisiana experiments (2, 4, 5, 6). The repeatability figures found in this experiment are in line with those of 0.42 and 0.48 reported (2) for studies made of data gathered during the 8 warmer observation days for 1944 and 1945, respectively. It seems probable that the results in the present experiment would have yielded higher estimates of repeatability had data for only the 8 warmer days been included.

Body temperature—repeatability. It has not proved practical to take rectal body temperatures of cows while they are in the pasture. To do so would necessitate haltering and tying animals; this would excite cows, thus tending to accelerate body heat production and increase respiration rate and body temperature.

Body temperatures of the 20 experimental cows taken after the cows entered the milking barn averaged 103.4° F. for the 14-day period. Average repeatability, when computed as in table 2, was 0.288. This was in line with the values of 0.152 and 0.385 previously reported (2) for 1944 and 1945, respectively, when only the 8 warmer days were used.

Number of observations needed. As explained by Lush (1), increasing the number of observations or records on an animal causes a gradual reduction in the temporary variance and an increase in the proportion of the total variance, much of which is genetic, caused by permanent differences between animals. Recognition of this fact has caused a great increase in the use of lifetime records in the evaluation of milk and fat records in pedigrees. This same trend holds true for other measurements and observations used in appraising animals. However, there is a limit to the number of observations it is practical to use. This is particularly true if the repeatability (r) between single records is relatively high. With n representing the number of observations, the formula of $\frac{nr}{1+(n-1)r}$ as presented by Lush (1) is the fraction showing how much one can trust a cow's own average as an index of her real ability. In this case it would mean the best estimate of her real ability to tolerate heat based on respiration rate and body temperature appraisals.

In table 3 are shown various values of this fraction, which is labeled "permanent differences between cows," as determined for various numbers of records varying from 1 to 10. In these computations the value of r was taken as averaging 0.4 for respiration and 0.25 for body temperature.

As revealed in the table, there is a relatively large increase in the fraction representing permanent differences between cows when n, the number of observations, increased from 1 to 2, from 2 to 3, and from 4 to 5. For example, for respiration with n increasing from 1 to 2, the increase in permanent differences is from 0.40 to 0.57, or a net gain of 0.17. These respective gains for each one-unit increase in n are 0.09, 0.07, 0.04, and 0.03 with a gain of 0.02 or less for each unit increase in n beyond values of 6. The same trend takes place for body temperature, with the fraction due to permanent differences increasing from 0.25, to 0.40, 0.50, 0.57, 0.62, and 0.67 for the respective values of n between 1 and 7. Each increment change beyond this point is 0.03 or less.

It would appear from the size of these computed values that little gain in accuracy would result from taking more than six respiration or seven body temperature observations. Under such a program approximately 0.8 of the total variance found in respiration rates and 0.7 of that for body temperature would be caused by permanent differences between cows. A high percentage of these permanent differences would be caused by heritable factors, and any selection based on six or seven observations would be conducive to more accuracy and faster progress in developing cattle more highly tolerant to heat than where fewer than this number of observations are made.

TABLE 3

Relationship between number of observations and portion of variance due to permanent differences between cows

No. of records	For respiration		For body temperature	
	Portion of variance due to permanent differences between cows*	Gain from preceding value	Portion of variance due to permanent differences between cows*	Gain from preceding value
1	0.40		0.25	
2	0.57	0.17	0.40	0.15
3	0.66	0.09	0.50	0.10
4	0.73	0.07	0.57	0.07
5	0.77	0.04	0.62	0.05
6	0.80	0.03	0.67	0.05
7	0.82	0.02	0.70	0.03
8	0.84	0.02	0.73	0.03
9	0.86	0.02	0.75	0.02
10	0.87	0.01	0.77	0.02

* 0.40 was used as average repeatability (r) for respiration and 0.25 for body temperature. Various values due to permanent differences between cows were computed for number of observations (n) from 1 to 10 using the fraction, $\frac{nr}{1+(n-1)r}$, proposed by Lush (1) as the degree to which one can trust a cow's own record in selection.

SUMMARY AND CONCLUSIONS

Data gathered from observations made for 14 days on 20 milking Holstein cows were used in this study. Mean air temperatures at 2:45 p.m. during the experimental period averaged 88° F. and relative humidity 60 per cent. Respiration rates were determined on cows while they were in the shade within pasture and just prior to entering milking barn. Shortly after entering milking barn both respiration rates and rectal body temperatures were determined.

Results of this study show the following:

1. The 14-day mean respiration rates for cows while in pasture averaged 78.5 times per minute and increased to an average of 80.4 times per minute for counts made in barn. Thirteen of the 20 cows had higher average rates after entering the barn, while seven had higher average rates while in pasture.
2. Relative ranking of the ten highest cows on the basis of pasture observations included seven also ranking highest when barn observations were used. The highest (1st) and lowest (20th) ranking cows were the same in both cases.
3. Computation of the repeatability of a single respiration record of the same cow resulted in 0.366 for pasture observations and 0.446 for barn observations.
4. Repeatability for body temperature observations made in the barn was 0.288. The mean body temperature for the 20 Holstein cows during the 14-day period was 103.4° F.
5. Computations are presented which show how the use of several observations increases the variance due to permanent differences between cows, much of which is genetic. With six respiration records this portion of the variance increased from an average of 0.4 to 0.8, and with seven body temperature observations the increase was from the average of 0.25 to 0.7.

It is concluded that respiration observations taken in the barn are as reliable as and perhaps slightly more reliable than those taken in the pasture. The use of averages for observations plus those for body temperature made on approximately 6 or 7 relatively warm days should result in measurements that are relatively accurate for use in selecting cattle for a high degree of tolerance to heat.

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A STUDY OF PROCEDURES USED IN CONDUCTING THE BABCOCK TEST IN THE VARIOUS STATES

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This study was undertaken because it had been reported to the Committee on Testing Milk and Cream of the American Dairy Science Association that sampling and testing methods as expressed in the various state regulations were not uniform. This lack of uniformity frequently has resulted in misunderstanding concerning the readings of the Babcock test in verifying the fat content of shipments of milk and cream from one state to another. At the annual meeting of the American Dairy Science Association in 1946, a Subcommittee was appointed to study this problem. The results are reported in this paper.

PROCEDURE

A form was prepared by members of the Subcommittee and the Committee on Testing Milk and Cream. This form included each technic in sampling and testing which might have a bearing on the accuracy of the Babcock test. To obtain the desired information, each state department of agriculture, board of health, or other responsible regulatory agency was requested to forward a copy of the laws and regulations pertaining to the sampling and testing of milk by the Babcock method or other approved methods. In some instances, where state laws were very incomplete, recourse was made to experiment station bulletins which were forwarded by the state regulatory agency concerned. In these instances, it generally is the practice of the agency to follow the experiment station recommendations, which thus have the same effect as a state regulation. After the data were compiled, a copy was returned to the proper state agency for verification. The final revision is correct, as nearly as possible, as of June 15, 1947.

SUMMARY OF REQUIREMENTS OF ALL STATES RELATING TO THE BABCOCK TESTING OF WHOLE MILK

I. *Procedure Specified by Law*

A. No specifications: Alabama (1)

B. Specify Babcock test: (36)

C. Specify Babcock or other method: Ark., Calif., Fla., Ill., Mass., Miss., N.J., N.Y., Okla., Utah, Va. (11)

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[2, 4] Chairman and member, respectively, of Committee on Testing Milk and Cream, Manufacturing Section, ADSA.

II. *Weighing and Sampling Milk at Receiving Room*

A. Weigh can requirements
  1. No specifications: (27)
  2. Mechanical stirrers: Calif., Maine (2)
  3. Strainer: Conn., Del., Ind., Ky., N.C., R.I., Texas, Vt. (8)
  4. No strainers, partitions or divisions: Md., N.J., Pa. (3)
  5. Sufficient agitation: N.H., N.Y. (2)
  6. Check test to verify efficient mixing: Minn., Ohio (2)
  7. Either mechanical agitator or stir each can: Wash. (1)
  8. Straight sided: W.Va. (1)
  9. Must be approved: Mont., Oreg. (2)

B. Mixing or stirring before sampling
  1. No specifications: (12)
  2. "Thoroughly mixed": Ala., Ark., Colo., Ill., Maine, Md., Mass., Mich., Nebr., N.Y., N.C., Tenn., Wis. (13)
  3. Representative: Calif., Del., Kans., Mont., N.D. (5)
  4. Adequate: Conn. (1)
  5. Pour back and forth or stir: Iowa, Ky., Nev., Oreg. (4)
  6. Stir 30 sec.: Minn., Texas (2)
  7. Contents of weigh tank or container shall be agitated: N.J. (1)
  8. Butterfat to be uniformly distributed: La., Ohio (2)
  9. Stir thoroughly each container: Pa., Wash. (2)
  10. Vigorous stirring: S.C. (1)
  11. Advisable to stir can: W.Va. (1)
  12. In such manner as to secure fair and average samples: Ariz. (1)
  13. Immediately after dumping: Mo. (1)
  14. Poured through strainer: Ind., Vt. (2)

C–1. Frozen milk procedure
  1. No specifications: (25)
  2. "Shall not be sampled": Conn., Del., Ind., Ky., N.Y., N.C., R.I., Tenn., Wash. (9)
  3. Thawed slowly and thoroughly mixed: Colo., Ill., Maine, Md., Mass., Mich., Minn., N.H., N.J., Vt., Va., W.Va. (12)
  4. Should not be sampled unless special precautions are taken: Ohio, Pa. (2)

C–2. Churned milk procedure
  1. No specifications: (24)
  2. "Shall not be sampled": Conn., Del., Ind., Ky., La., Maine, Md., Mass., Mich., Minn., N.H., N.J., N.Y., N.C., R.I., Tenn., Vt., Wash. (18)
  3. Warm gradually: Mo., Mont., Va., W.Va. (4)
  4. Should not be sampled unless special precautions are taken: Ohio, Pa. (2)

D. Sampler license required
  1. Required: Ark., Calif., Colo., Conn., Del., Ill., Kans., La., Md., Mich., Minn., Mo., Mont., N.J., N.Mex., N.Y., N.C., Ohio, Pa., R.I., Texas, Vt., Va., Wash., W.Va. (25)
  2. Not required: Ala., Ariz., Fla., Ga., Idaho, Ind., Iowa, Ky., Maine, Mass., Miss., Nebr., Nev., N.H., N.D., Okla., Oreg., S.C., S.D., Tenn., Utah, Wis., Wyo. (23)

III. *Care of Composite Samples*

A. Preservative used
  1. Not specified: (16)
  2. Suitable: Ariz., Ark., Ky., Mass., Oreg., R.I., Texas (7)
  3. Solid not to exceed 1% by wt.; liquid not to exceed 2 drops fl. oz.: Calif. (1)
  4. 1 part per 1000 formaldehyde or 1 mercuric chloride tablet: Colo. (1)
  5. Approved: Conn., N.J., N.C. (3)
  6. Mercuric chloride: Del., Ill., Ind., La., Maine, Md., Mo., Mont., N.H., Wash., W.Va. (11)
  7. Mercuric chloride, potassium dichromate, or formaldehyde: Mich., Minn., Ohio, Pa., S.C., Va. (6)
  8. 0.3 g. mercuric chloride or equivalent: N.Y., Wis. (2)
  9. 0.5 g. mercuric chloride: Vt. (1)

B. Maximum period of composite sampling
  1. Not specified: (20)
  2. 16 days: Ark., Del., Ky., La., Maine, Md., Mass., Minn., N.H., N.J., N.Y., Pa., R.I., Texas, Vt., Wash., W.Va. (17)
  3. 15 days: Ind., Oreg., Va. (3)
  4. 14 days: Mich., Mo., Mont. (3)
  5. 11 days: Conn., N.C. (2)
  6. 8 days: Calif. (1)
  7. Twice a month: Ohio (1)
  8. Weekly: Tenn. (1)

C. Quantity of aliquot required
  1. Not specified: (32)
  2. Not less than 17 cc.: Conn., N.C. (2)
  3. 5 to 10 cc.: Kans. (1)
  4. At least 10 cc.: Ind., Ky., Minn., N.Y., R.I., Texas, Vt., Wash. (8)
  5. Total of 75 cc. suggested: Ohio (1)
  6. 10 to 12 cc. satisfactory: W.Va., (1)
  7. 0.5 oz.: Ark. (1)

   8. Sufficient for 4 tests: Md. (1)
   9. 1 oz.: Mont. (1)

D. Size of sample bottle
   1. Not specified: (27)
   2. At least 2 oz.: Calif., Minn. (2)
   3. Not less than 4 oz.: Conn. (1)
   4. Not less than 8 oz.: Ark., Del., Ind., N.Y., Ohio, Tenn., Wash., Wis. (8)
   5. 8 or 16 oz.: Ky., La. (2)
   6. Preferably 8 oz.: Md., N.H., Vt. (3)
   7. 4 oz. or 8 oz.: Mass. (1)
   8. Sufficient for 3 Babcock tests: La., N.J. (2)
   9. 6 oz.: W.Va. (1)
   10. Sufficient for period: Mont. (1)

E. Method for taking
   1. Not specified: (15)
   2. Representative or proportionate: Ala., Ariz., Calif., Colo., Del., Ill., Ind., Kans., Ky., Maine, Md., Mass., Mich., Minn., N.J., N.C., Pa., Tenn., Texas, Va., Wis. (21)
   3. With thief, pipette or similar sampler: Ark., Iowa, Ohio, Vt., W.Va. (5)
   4. From approved weigh can: Conn. (1)
   5. Automatic samplers must be approved: N.Y. (1)
   6. Milk thief or approved weigh tanks: R.I. (1)
   7. With dipper: La., Mont., N.H., Wash. (4)

F. Mixing at time of addition of aliquot
   1. Not specified: (22)
   2. Gentle rotary motion: Calif., Conn., Del., Ind., Ky., Mass., N.H., N.J., N.Y., Ohio, R.I., Texas, Vt., Va., Wis. (15)
   3. Shaken to insure thorough mixing: Colo., Mich., Minn. (3)
   4. Rotary motion and invertance: La., Maine (2)
   5. Should not be rotated: Ill., Md. (2)
   6. Gently tipped: Wash., W.Va. (2)
   7. Mixed: Ark., Mont. (2)

G. Storage conditions
   1. Not specified: (18)
   2. Cared for or preserved in such a manner to insure accurate test thereof: Ariz., Mass., W.Va. (3)
   3. 60° F. or below: Calif., Md. (2)
   4. 32–40° F.: Conn. (1)
   5. 40–50° F.: Del., Idaho, N.Y., Ohio, Wash. (5)
   6. Protected against high temp.: Ind., Minn., Oreg. (3)

7. Cool, clean, sanitary place: Ill., Kans., N.Mex., R.I., Texas (5)
8. Cool, away from strong light: Ky., Maine, Mich., N.H., N.C., Vt. (6)
9. Below 55° F.: N.J. (1)
10. As cold as practicable—never above 70° F.: Wis. (1)
11. Preferably refrigerated: Ark. (1)
12. 35–55° F.: La. (1)
13. Protected from extreme temp.: Mont. (1)

H. Period held after testing
  1. Not specified: (12)
  2. 24 to 48 hours: Ark. (1)
  3. 5 days: Calif., Ill., Mass., R.I., Wis. (5)
  4. 10 days: Colo., Del., Mo., N.Y., Pa., Vt., Wash. (7)
  5. Until last sample has been added to succeeding composite: Conn., La., Mont., N.J. (4)
  6. 3 days: Idaho, Ky. (2)
  7. 2 days: Ariz., Iowa, Miss., Oreg. (4)
  8. 1 day: Kans., Mich., Minn., Nev., Texas (5)
  9. 7 days: Ind., Maine, Md., N.C. (4)
  10. 4 days: N.H., Tenn. (2)
  11. "Period of time specified by Law": Ohio (1)
  12. 12 days: W.Va. (1)

IV. *Preparing Fresh Samples for Testing*

A. Tempering
  1. Not specified: (33)
  2. In water bath: Colo., N.Y. (2)
  3. Mix thoroughly: Conn. (1)
  4. Adjust to 60–65° F.: Del. (1)
  5. Adjust to 59–68° F.: Maine, Mo., Utah (3)
  6. Adjust to 55–65° F.: Md. (1)
  7. Warm to about 70° F.: Mass., Texas, Vt. (3)
  8. Water bath for 50 minutes at 60–70° F.: S.D., Wash. (2)
  9. Heat to 68–70° F.: La., Mont. (2)

B. Temperature at time of sampling
  1. Not specified: (27)
  2. 65–75° F.: Colo. (1)
  3. 60–68° F.: Conn. (1)
  4. 60–65° F.: Del. (1)
  5. 55–70° F.: Ind., Mich. (2)
  6. 60–70° F.: Ky., Mont., N.J., Ohio, S.D., Wash. (6)
  7. 68° F.: La., Maine, Mo., Utah (4)

8. 55–65° F.: Md. (1)
9. About 70° F.: Mass., Texas, Vt. (3)
10. 56–68° F.: Minn. (1)
11. 60–100° F. (prefer 60–70° F.): N.Y. (1)

C. Amount of sample
1. Not specified: (29)
2. 17.6 cc.—blow last drop: Colo., Conn., Del., Ind., Ky., La., Maine, Md., Mass., Mo., N.J., N.Y., Ohio, S.D., Texas, Utah, Vt., Wash.. (18)
3. 17.5 cc.: Mont. (1)

V. *Preparing Composite Samples for Testing*

A. Tempering
1. Not specified: (18)
2. Not to exceed 90° F.: Ark. (1)
3. Not to exceed 95° F.: Calif., Wis. (2)
4. Gradually heated to 100° F.: Colo., Del., Ind., Maine, Mich., Minn., N.H., Pa., R.I., S.D., Vt., Wash. (12)
5. 95–105° F. at risk of tester: Conn. (1)
6. 30-min. bath at 90° F.: Kans. (1)
7. 95–100° F.: Md., N.J., N.Y. (3)
8. Not to exceed 100° F.: Mass. (1)
9. Heat to 110° F.: N.C., Tenn., Va. (3)
10. No higher than 104° F.: Ohio (1)
11. 85–110° F.: W.Va. (1)
12. Room temperature: Ill. (1)
13. Heated to 100° F.: La., Mo., Mont. (3)

B. Temperature of bath
1. Not specified: (22)
2. 90° F.: Ark., Calif., Kans. (3)
3. 122° F.: Conn. (1)
4. 105° F.: Del., Ky., Mo., Ohio (4)
5. Hot water: Colo., Ind., Tenn. (3)
6. 110° F.: Md., Nebr., S.D., Texas, Vt., Va., Wash. (7)
7. 100° F.: La., Minn., Mont., N.H., Pa. (5)
8. 120° F.: N.Y. (1)
9. 85–110° F.: W.Va. (1)
10. 100–110° F.: N.J. (1)

C. Mixing or stirring before pipetting
1. Not specified: (17)
2. Lumps thoroughly dissolved: Ariz. (1)
3. Pour back and forth: Ark., Conn., Del., Iowa, Kans., Ky., Maine,

Md., Mich., Minn., Mont., N.J., N.Y., Ohio, Pa., S.D., Wash., Wis. (18)

4. By stirring or pouring back and forth: Ind., Tenn. (2)
5. Gentle rotary motion: Mass., Mo. (2)
6. Mix: N.C., R.I. (2)
7. Do not shake vigorously—"Use policeman": Texas (1)
8. Thoroughly mixed: Ill., La. (2)
9. Use brush to loosen cream and pour 3 times: N.H., Vt. (2)
10. Pour back and forth; use policeman: Calif. (1)

D. Temperature at time of pipetting

1. Not specified: (14)
2. 50–70° F.: Ariz., N.C. (2)
3. 70–95° C.: Calif. (1)
4. 65–75° F.: Colo. (1)
5. 60–68° F.: Conn. (1)
6. 55–65° F.: Md. (1)
7. 100° F.: Del., Ind., La. (3)
8. 60–70° F.: Iowa, Mont., N.J., Ohio, Oreg., S.D., Wash. (7)
9. 90° F.: Kans. (1)
10. 60–100° F.: N.Y. (1)
11. 68° F.: Maine, Minn., Mo. (3)
12. About 70° F.: Ill., Mass., R.I., Vt., Wis. (5)
13. 55–70° F.: Mich., Va. (2)
14. 50–100° F.: Pa. (1)
15. Not over 110° F.: Tenn. (1)
16. Warm: Texas (1)
17. 85–110° F.: W.Va. (1)
18. 95–100° F.: Ky., N.H. (2)

E. Amount of sample

1. Not specified: (10)
2. 18 g. or 17.6 cc.: Ark., Kans., Minn., Mont., Nebr., N.H., Oreg., Va., Wyo. (9)
3. 17.6 cc.'s—blow last drop: Calif., Colo., Conn., Del., Ind., Iowa, Ky., Mass., Mich., Mo., Ohio, Pa., S.D., Texas, Wash. (15)
4. 17.6 cc.'s: Ill., Nev., N.J., N.C., R.I., Tenn., Vt., Wis. (8)
5. To deliver 17.5 ml. or T.C. 17.6 ml. at 68° F.: Maine, N.Y., W.Va. (3)
6. 17.5 ml.—drain 5 sec.—blow last drop: Md. (1)
7. 17.5 cc.'s: Ariz., La. (2)

VI. *Testing*

A. Tester license required

1. Yes: (40)

2. Not specified: Ga., Maine, Okla., S.C., S.D., Utah, Wis., Wyo. (8)

B. Specific gravity of acid
 1. Not specified: (14)
 2. Proper strength: Ariz. (1)
 3. 1.8253 to 1.8263: Colo. (1)
 4. 1.82–1.83 at 68° F.: Ark., Conn., Ill., Md., Minn., Mo., Ohio, Texas, Utah (9)
 5. 1.82–1.83 at 60° F.: Del., Maine, Pa. (3)
 6. 1.82–1.83: Calif., Ind., Iowa, La., Mass., Mich., Mont., Nebr., N.H., N.C., Wis. (11)
 7. 1.82–1.83 at 59° F.: N.J., R.I., S.C., Tenn., Vt., Va. (6)
 8. 1.820–1.825 at 68° F.: N.Y. (1)
 9. 1.82: W.Va. (1)
 10. 1.825–1.830 at 60° F.: Wash. (1)

C. Amount of acid
 1. Not specified: (11)
 2. About 17.5 cc.: Ark., La., Mo. (3)
 3. 17.5 cc.: Colo., Conn., Del., Ill., Ind., Iowa, Kans., Maine, Md., Mass., Mich., Minn., Mont., N.H., N.J., N.C., R.I., S.D., Tenn., Texas, Utah, Vt., Va., Wash., W.Va., Wis., Wyo. (27)
 4. Approximately 17.6 cc.: Nebr. (1)
 5. Variable to secure clear fat column: Ky., N.Y., Pa. (3)
 6. 15–17.5 cc.: Ohio (1)
 7. 17.0 cc.: S.C., Va. (2)

D. Temperature of acid
 1. Not specified: (13)
 2. 70° F.: Calif., La., Mass., Nebr., N.H., R.I., Vt. (7)
 3. 65–75° F.: Colo. (1)
 4. 60–68° F.: Conn. (1)
 5. 60–65° F.: Del., Wis. (2)
 6. 55–70° F.: Ind., Mich., Va. (3)
 7. 60–70° F.: Iowa, Mont., N.Y., N.C., Ohio, S.D., Tenn., Texas, Wash., W.Va., Wyo. (11)
 8. 59–68° F.: Maine, Mo., S.C., Utah (4)
 9. 55–65° F.: Md. (1)
 10. 65–68° F.: Minn. (1)
 11. 50–100° F.—(50–70° preferred): Pa. (1)
 12. 65–70° F.: Ark., Ill. (2)
 13. 68–72° F.: N.J. (1)

E. Method of adding acid
 1. Not specified: (20)

2. Hold bottles at angle and rotate: Colo., Ind., Iowa, Md., Minn., Mont., N.H., N.C., S.D., Wash., W.Va. (11)
3. Down neck of bottle—preferably not all at one time: Conn., Mo., N.J., Texas (4)
4. Hold bottle at angle: Del., Ky., Maine, Mass., Nebr., N.Y., Ohio, Pa., S.C. (9)
5. Rinse down milk—allow to stand 1–3 min. before cent.: Vt. (1)
6. Small portions, washing curd from neck: Ill. (1)
7. All at one time: La. (1)
8. Add slowly: Mich. (1)

F. Centrifuging procedure
1. Not specified: (10)
2. 5 min., 2 min., and 1 min.: Ariz., Conn., Del., Ind., La., Mich., Mont., Nebr., N.J., Ohio, S.D., Va., Wis. (13)
3. 5 min.—place in water bath 5 min.—cent. 2 min., 1 min.: Ark., Kans. (2)
4. 5 min., 2 min., and 1 min. at proper speed: Calif., Colo., Ill., Ky., Maine, Md., Minn., Mo., N.H., N.Y., N.C., Pa., R.I., S.C., Tenn., Texas, Utah, W.Va., Wyo. (19)
5. 4 to 5 min.—1 min.: Iowa (1)
6. 5 min.—mix thoroughly—2 min. to 1 min.: Mass., Vt. (2)
7. 5 min. or more for 1st; 2 min. for 2nd; 1 min. or more for 3rd: Wash. (1)

G. Speed centrifuge
1. Not specified: (11)
2. Standard: Ariz., Ark., Calif., Colo., Conn., Del., Ill., Ind., Iowa, Ky., La., Maine, Md., Mass., Mich., Minn., Mo., Mont., Nebr., N.H., N.J., N.Y., N.C., Ohio, Oreg., Pa., R.I., S.C., Tenn., Texas, Utah, Vt., Wash., W.Va., Wis. (35)
3. 14″–900 RPM; 20″–750 RPM: Kans. (1)
4. 12″–1000 RPM; 18″–800 RPM: Va. (1)
5. Belt driven not permitted: W. Va. (1)

H. Temperature of centrifuge
1. Not specified: (26)
2. 140–165° F.: Calif. (1)
3. 131° F.: Conn., Minn., Mo., Ohio, S.C., Utah, W.Va. (7)
4. Heated: Wis. (1)
5. 120° F.: Ky. (1)
6. 130–140° F.: Ark., Ill., Md., N.C., S.D., Wash. (6)
7. Unheated if room is warm; heated if room is cold: Mich. (1)
8. 135–150° F.: N.Y. (1)
9. 140–150° F.: La. (1)
10. About 140° F.: Del., N.H., Vt. (3)

I. Kind of water
  1. Not specified: (15)
  2. Soft: Ark., Colo., Conn., Del., Ind., Iowa, Ky., Maine, Md., Mass., Mich., Minn., Mo., N.H., N.J., N.Mex., N.Y., N.C., Ohio, Pa., R.I., S.C., S.D., Tenn., Texas, Utah, Vt., Va., Wash., W.Va., Wis., Wyo. (32)
  3. Tap water, preferably soft: La. (1)

J. Temperature of water
  1. Not specified: (12)
  2. Hot: Ark., Iowa (2)
  3. 140–165° F.: Calif., Mich. (2)
  4. 180–212° F.: Colo., Kans., Nebr., N.Mex. (4)
  5. 140° F.: Conn., Del., Ill., Ind., Maine, Minn., Mo., Mont., N.J., N.C., Ohio, Pa., S.C., Texas, Utah, Wis., Wyo. (17)
  6. 135–200° F.: Ky., Tenn. (2)
  7. Above 160° F.: La., Mass., N.H., R.I., Vt. (5)
  8. 150° F. (200° F. if unheated tester is used): N.Y. (1)
  9. 140–150° F.: S.D., Wash. (2)
  10. Above 150° F.: W.Va. (1)

K. Temperature of bath
  1. Not specified: (6)
  2. 130–140° F.: Ariz., Ark., Calif., Ill., Iowa, La., Mass., Minn., Mo., Mont., Nev., Oreg., Texas, Va., Wyo. (15)
  3. 130° F.: Colo. (1)
  4. 131–140° F.: Conn., Maine, S.C., Utah (4)
  5. 135–140° F.: Del., Ind., Ky., Md., Mich., N.H., N.J., N.Y., Ohio, R.I., Tenn., Vt., Wash., Wis. (14)
  6. Not less than 135° F.: Idaho, Kans. (2)
  7. 125–140° F.: Miss. (1)
  8. 120–135° F. (law), 135–140° F. (Circ.): Nebr. (1)
  9. 120° F.: N.Mex. (1)
  10. 135–145° F.: N.C. (1)
  11. 135–140° F. (law), 130–140° F. (Circ.): Pa. (1)
  12. 130–145° F.: W.Va. (1)

L. Time in bath
  1. Not specified: (10)
  2. 5 min.: Ark., Colo., Del., Ind., Ky., La., Maine, Mass., Nebr., N.H., N.J., N.Y., Ohio, Pa., R.I., S.D., Tenn., Texas, Vt., Va., Wash., W.Va. (22)
  3. 10 min.: Ariz., Calif., Kans., Mont., N.Mex. (5)
  4. Not less than 3 min.: Conn., Md. (2)
  5. Several min.: Iowa (1)

6. 3 min. by law, 5 min. by ext. bul.: Mich. (1)
7. 7–8 min.: Minn. (1)
8. Until equilibrium has been reached: Ill., Mo., S.C., Utah (4)
9. 20 min.: N.C. (1)
10. 3 to 10 min.: Wis. (1)

M. Method of reading
   1. Not specified: (11)
   2. Extreme bottom to extreme top: Ark., Calif., Colo., Conn., Del., Ind., Iowa, Kans., Ky., Mass., Mich., Minn., Mo., Mont., Nebr., N.H., N.J., N.Y., Ohio, Pa., R.I., S.C., S.D., Texas, Utah, Vt., Va., Wash., W.Va., Wis., Wyo. (31)
   3. Lowest to highest point (should not be read if fat column is outside graduation marks): Maine (1)
   4. Lower line of separation to top of fat column: Nev., N.C. (2)
   5. Dividers always should be used: Ill., La., Tenn. (3)

N. Disposition of fractions
   1. Not specified: (36)
   2. To nearest 1/10 graduation: Ill., Ind., La., Maine, Md., Mont., N.J. (7)
   3. Do not record fractions: N.Y. (1)
   4. To nearest lower 1/10: Minn., Mo., W.Va. (3)
   5. To nearest higher 1/10: N.H. (1)

VII. *Glassware*

A. Specifications
   1. Not specified: (21)
   2. Standard specifications included: Calif., Conn., Ind., Ky., Md., Mass., Minn., Nebr., N.Y., N.D., Oreg., S.C., Texas, Wash. (14)
   3. Standard specifications but not included: Ariz., Del., Idaho, Kans., Mo., N.H., Ohio, Utah (8)
   4. 8 or 10% bottle: Ill., Mont., Vt., Va. (4)
   5. Gives details for calibrating: N.C. (1)

B. Certified or retested
   1. Not specified: (21)
   2. Required: Ariz., Calif., Conn., Del., Idaho, Ind., Kans., Ky., Maine, Md., Mass., Mich., Minn., Nebr., N.H., N.J., N.Y., Oreg., Pa., R.I., Tenn., Vt., Va. (23)
   3. Sealed: La., Miss., N.Mex., Wash. (4)

DISCUSSION

The results of this survey indicate considerable variation in specifications for sampling and testing technics in conducting the Babcock test. Technics approved by some states are prohibited by others. In the opinion of the committee, some of the specifications are wrong; others are unnecessary. The fact that a number of specifications are omitted by the majority of the states is an indication of the lack of recognition of some of the important aspects of the Babcock test and of lack of uniformity in use of the test.

CONCLUSIONS

It is the recommendation of this committee that a standardized procedure in connection with weighing, sampling, and care of composite samples and the operation of the Babcock test be determined and approved by the American Dairy Science Association.

THE RESISTANCE OF *MICROCOCCUS FREUDENREICHII* IN LABORATORY HIGH-TEMPERATURE-SHORT-TIME PASTEURIZATION OF MILK AND ICE CREAM MIX

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In the course of some preliminary laboratory experiments dealing with the high-temperature-short-time (HTST) pasteurization of ice cream mix, it was desirable to determine the times and temperatures required to insure the destruction of pathogenic bacteria in this product. Studies of this nature usually have been made using a non-pathogenic test organism having somewhat greater resistance to heat than the most heat-resistant pathogen. This procedure avoids certain hazards which are encountered when pathogens are used, particularly in plant studies, and provides a margin of safety for the destruction of pathogens.

Fuchs (3), during a study of the sterilization of milk utensils by heat and chlorine in 1932, isolated a particularly heat-resistant strain of *Escherichia coli* which was used as an index of satisfactory sterilization. This culture has since been used as a test organism by many investigators studying pasteurization processes and is identified as the U. S. Public Health Service strain of *E. coli* (no. 3 U). Myers and Sorensen (6) used this organism to determine the times and temperatures required to destroy the *Escherichia* and *Aerobacter* groups of organisms in ice cream mix. Frank *et al.* (2) also used this culture as the test organism in a study of the bactericidal treatment of milk cans in hot air cabinets. Holland and Dahlberg (5), in an investigation of the effect of time and temperature of pasteurization on certain properties and constituents of milk, and Hening and Dahlberg (4), in a similar study on cream, used this culture of *E. coli* as a test organism for proper pasteurization.

It was planned to use the *E. coli* (no. 3 U) in the present investigation. However, a recent survey in this laboratory of transfers of this organism from various sources failed to reveal any strains which possessed the degree of heat resistance which was found in earlier studies. In attempts to find another organism which could be used as a test organism, a heat-resistant micrococcus was isolated and found to possess characteristics desirable for this study.

EXPERIMENTAL PROCEDURE

During an experiment dealing with the heat resistance of *E. coli* (no. 3 U) in milk, small red colonies which were not typically coliform developed

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on some of the desoxycholate lactose agar (BBL) plates after 48 hours at 37° C. These colonies appeared on plates poured from samples of pasteurized milk which had been inoculated with *E. coli* (no. 3 U) and heated at 140° F. for 50 minutes, but not on plates poured from the same milk heated at 140° F. for 55 minutes. Studies on a pure culture from a typical colony showed it to be a micrococcus which tentatively was identified as *Micrococcus freudenreichii* (no. MS66). In 48 hours at 37° C. on desoxycholate lactose agar, colonies of this organism were pink to red in color and about 1 mm. in diameter. Since the culture apparently had the resistance formerly possessed by *E. coli* (no. 3 U) and since it could be grown on a selective medium, its heat resistance and usefulness as a test organism in pasteurization methods were studied.

Stock and test cultures of *M. freudenreichii* were carried on agar slants of a medium containing 1.0 g. of yeast extract (BBL), 0.5 g. of NZ case (Sheffield), 0.5 g. of glucose, 0.4 g. of K_2HPO_4, 0.1 g. of KH_2PO_4, 1.5 g. of agar, and 100 ml. of distilled water. Adjust to pH 7.0, if necessary, before sterilization at 15 lb. steam pressure for 20 minutes.

Weekly transfers of the culture were prepared and incubated at 37° C. for 24 hours. These stock cultures were stored in the refrigerator from 1 to 10 days, and during this storage period were used to inoculate test cultures. The test culture, grown on a slant of medium of the above composition, was incubated at 37° C. for 24 hours and used immediately for a pasteurization test. More uniform resistance of the culture was obtained if the test cultures were prepared from a stock culture which had been refrigerated at least 1 day and preferably not more than 10 days.

The growth of the test culture was washed completely from the slant (5 ml. of medium slanted in a 16-mm. culture tube), suspended in 40 to 50 ml. of sterile distilled water, shaken vigorously 100 times, and then filtered through sterile no. 12 folded Whatman filter paper. This suspension was kept at room temperature and used for the tests within 45 minutes after its preparation. This suspension in 0.05-ml. quantities was added to 5 ml. of sample and usually gave 1 to 2 million cells per milliliter of sample.

Milk used in this study was pasteurized and contained 4.6 per cent butterfat. The ice cream mix was obtained by melting (at about 50° C.) vanilla ice cream as purchased in pint packages. The average composition of this mix was 12 per cent fat, 11 per cent milk solids-not-fat, 14 per cent sucrose, and 0.25 per cent stabilizer, with the reaction adjusted to pH 6.7.

In order to avoid the variations which occur in the time for heating to the different temperatures, milk or ice cream mix first was tempered to the temperature under test and then inoculated. The method used was modeled somewhat after the one used by Fuchs (3), who eliminated heating-up times in his pasteurization studies by inoculating a tube of tempered milk with a drop of test culture. In the present study, 5 ml. of milk or mix was placed in a sterile 120 × 20 mm. screw-cap glass vial, and then a rubber diaphragm stopper inserted in the vial to replace the cap. All vials thus were practically airtight. In the skirt of the stopper was placed a cotton plug which was kept in place until the tube was inoculated with the test culture. Then the material was tempered to the proper temperature, as shown by a test thermometer inserted through a rubber stopper and placed in a separate vial containing 5 ml. of the material under test. Heating was done in a thermostatically controlled water bath. The temperature of the material under test was controlled to about ± 0.25° F. Tubes were immersed in the bath to the shoulder of the tube, which made the water level about 3.5 inches above the level of the material under test. Unheated controls usually were inoculated for each temperature, except in the HTST experiments, where the interval from one temperature to another was very short. Uninoculated controls also were run to determine if the uninoculated material had any interfering organisms present.

When the material under test had been tempered properly, 0.05 ml. of the culture suspension was inoculated into it through the rubber stopper by means of a 1-ml. tuberculin syringe equipped with a 2-inch 20-gauge hypodermic needle. This permitted instantaneous inoculation and mixing of the test material with an accurate volume of the suspension, and the inoculum could be directed entirely into the test material with none going on the sides or top of the vial. The inoculum usually lowered the temperature of the test material 0.3 to 0.4° F. To compensate for this, the material under test was tempered 0.3 to 0.4° F. higher than the temperature under test. Where extensive holding was done at a given temperature, the bath was adjusted to hold at the temperature under test after inoculating a series of vials at intervals of 30 seconds. For short holding periods at high temperatures, no adjustment was made to lower the temperature of the bath since considerable time was required to equalize the temperature of the milk or ice cream mix with that of the surrounding bath. When the holding time was less than two minutes, the vials were shaken continuously during this period; for longer holding times they were shaken intermittently every 1 to 2 minutes. At the end of the holding period, the vial was immersed immediately in ice water and shaken to cool the sample rapidly. Samples were refrigerated until plated.

The pasteurized samples usually were plated with 1 ml. of the sample and in duplicate using desoxycholate lactose agar as the plating medium. When less than 1 ml. of sample was plated there was some indication that the medium had an inhibitory effect on the heated cells of *M. freudenreichii*. When plating ice cream mix, an overlay of medium (3 to 5 ml.) was used to prevent spreading growth of any surviving bacteria of this type. The plates were incubated at 37° C. for 2 days. An additional 24-hour incubation gave only an insignificant increase in count of the pasteurized samples.

RESULTS AND DISCUSSION

In table 1 the average times for 99.99 per cent destruction in milk at various temperatures are given for *M. freudenreichii* (no. MS66) and *E. coli* (no. 3 U), the data for the latter being taken from the studies of Holland and Dahlberg (5) and Hening and Dahlberg (4). The data show that the two cultures had very similar degrees of heat resistance, although the methods of pasteurization differed somewhat. In view of these results and the ability of the culture to grow on a selective medium, *M. frudenreichii* appeared to be a desirable test organism to use in these studies since a culture of *E. coli* (no. 3 U) having satisfactory heat resistance was not available.

TABLE 1

The thermal death times (99.99 per cent destruction) at various temperatures of Escherichia coli (no. 3 U) and Micrococcus freudenreichii (no. MS66) in milk

Temperature	*E. coli* (no. 3 U)		*Micrococcus freudenreichii* (no. MS66)
	Holland and Dahlberg (5)	Hening and Dahlberg (4)	
(*°F.*)			
140	50 min.	. .	55 min.
145	15 min.*	20 min.	17.5 min.
150	4 min.	4 min.	5 min.
155	1.5 min.	1.5 min.	1 min.
160	17.5 sec.	20 sec.	15 sec.
165	7.5 sec.	3 sec.*	4 sec.
170	0 sec.	2–3 sec.*	0 sec.

* No data given for shorter holding times.

The heat resistance of *M. freudenreichii* in ice cream mix and milk was determined, in most instances from a number of tests, as shown in table 2. Individual experiments at times gave thermal death times slightly above or below the averages shown. The thermal death times of the test culture in milk and ice cream mix also were plotted graphically, as shown in figure 1. The thermal death time of the test organism at a given temperature was about two to four times longer in ice cream mix than in milk, the difference being greater at the lower temperatures and becoming smaller with an increase in temperature.

It is reasonable to expect that the thermal death times found for *M. freudenreichii* in ice cream mix represent, at the least, the minimum times and temperatures which would eliminate pathogenic bacteria in this product. The problem of establishing the margin of safety which must be added for satisfactory HTST pasteurization can be solved by determining the times and temperatures which will give as effective pasteurization as that

TABLE 2

The destruction of Micrococcus freudenreichii (no. MS66) at various times and temperatures in milk and ice cream mix

Temperature	Time	Milk		Ice cream mix	
		Destruction	No. of tests	Destruction	No. of tests
(°*F.*)	(*min.*)	(%)		(%)	
140	40	*	6		
	45	*	6		
	50	99.42	5		
	55	99.99	6		
	60	99.99	1		
	65	99.99	1		
145	10	99.93	3		
	12.5	99.94	1		
	15	99.98	4		
	17.5	99.99	1		
	20	99.99	4		
150	2	*	3		
	3	99.96	3		
	4	99.98	4		
	5	99.99	1	*	1
	6	99.99	4		
	10			99.97	2
	12.5			99.98	1
	15			99.99	2
	17.5			99.99	1
	20			99.99	2
155	0.5	99.95	5		
	1.0	99.99	6	99.97	2
	1.5	99.99	6		
	2.0			99.99	4
	3.0			99.99	1
	4.0			99.99	5
	(*sec.*)				
160	5	*	4		
	10	99.97	4	*	5
	12.5	99.94	1		
	15	99.99	3		
	20	99.99	2	*	4
	30			99.97	4
	45			99.99	4
	60			99.99	5
165	1	99.93	2		
	2	99.95	1		
	2.5			99.81	2
	3	99.98	2		
	4	99.99	1		
	5	99.99	2	99.96	2
	7.5	99.99	2	99.97	2
	10			99.97	2
	12.5			99.99	2
	15			99.99	2
	20			99.99	2
170	0	99.99	4	*	3
	1	99.99	4	99.99	3
	2	99.99	4	99.99	3
	3			99.99	2

* Colonies too numerous to count in these samples.

given by 155° F. for 30 minutes, as specified in the Frozen Desserts Ordinance and Code (7). Since the present investigation shows a good margin of safety in the usual practice of pasteurization at 155° F. for 30 minutes, equivalent HTST pasteurization, whether established by a heat-resistant test organism, reduction in total bacterial count, or some other means, should have an equivalent margin of safety. Thus pasteurization at 180° F. for 19 seconds, as found by Dowd and Anderson (1) to be equivalent to 160° F. for 30 minutes in reducing the total bacterial count of ice cream mix, would afford a good margin of safety according to the results of the present study.

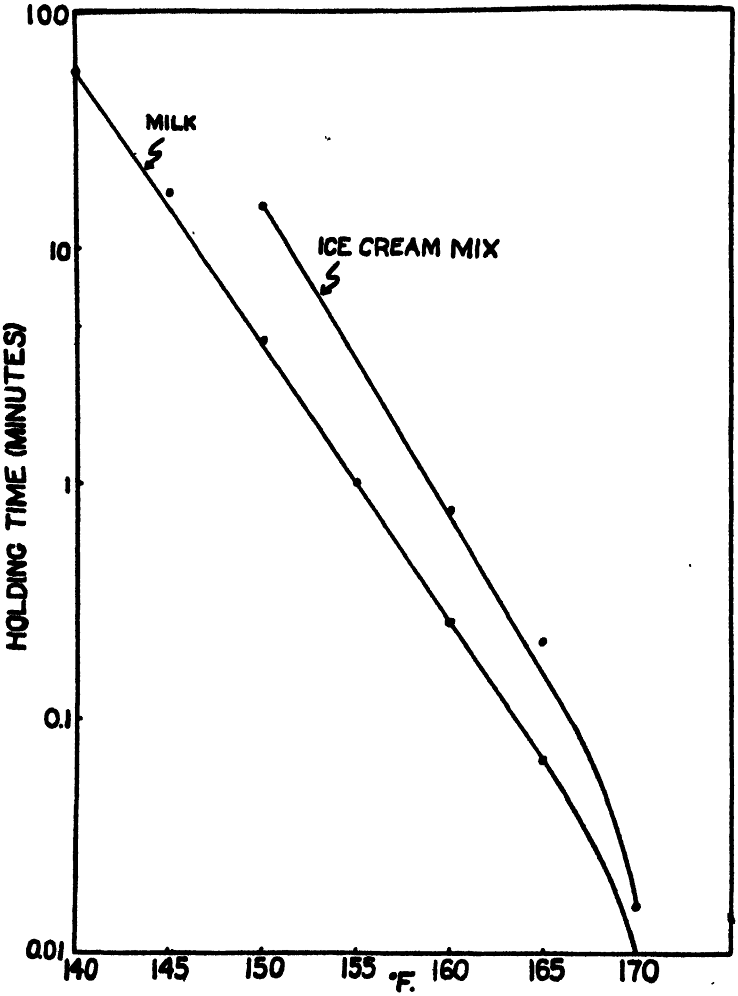

FIG. 1. The thermal death time (99.99 per cent destruction) of *M. freudenreichii* (no. MS66) in milk and ice cream mix. (Plotted semi-logarithmically.)

SUMMARY AND CONCLUSIONS

An organism tentatively identified as *M. freudenreichii* and designated strain no. MS66 was isolated and its heat resistance in milk and ice cream mix was studied. It was found to possess about the same degree of heat resistance in milk as was formerly possessed by the heat-resistant *E. coli* (no. 3 U), and was used as the test culture in laboratory pasteurization studies.

An improved laboratory pasteurization technique was devised for conducting HTST pasteurization tests which should give results similar to those obtained in commmercial HTST pasteurization units.

The length of time required for 99.99 per cent destruction of *M. freudenreichii* (no. MS66) at a given temperature was two to four times greater for ice cream mix than for milk, this difference decreasing with increased temperatures.

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CHANGES IN CELL VOLUME AND IN CONCENTRATION OF HEMOGLOBIN AND OF SEVERAL INORGANIC CONSTITUENTS OF THE BLOOD OF CALVES DURING EARLY POSTNATAL DEVELOPMENT[1]

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Knowledge of the composition of blood from normal dairy cattle under typical feeding and managemental practices is requisite to the diagnosis of many metabolic disorders. Considerable investigation has been directed toward ascertaining normal values for many of the blood constituents of adult cows in various stages of the reproductive cycle, but, with the exception of carotenoids and of vitamin A, little attention has been given to determinations of the changes that normally occur in the composition of the blood of calves during the critical periods of early postnatal development.

Limited data on the mineral constituents of the blood of young calves (1, 3, 9, 10, 15, 16, 17) indicate that following birth inorganic phosphorus of the blood increases while potassium decreases; whereas cell volume, calcium, and magnesium undergo no significant changes. In most of these studies small numbers of experimental subjects and infrequent analysis of the blood have yielded too few data to establish the normal trends.

The present investigation, a phase of a study also including the changes in the compostition of the blood of the parturient cow, was undertaken to provide information on the cell volume and the hemoglobin content of blood and the concentration of various inorganic constituents of the blood and of the serum of the calf during the normal transitory stages of early life.

PROCEDURES

The experimental subjects, 11 normal dairy calves representing the Ayrshire, the Hostein, and the Jersey breeds, were subjected to a typical herd regime. The dams of the calves had received standard barn rations and seasonal pasture during the terminal 6 to 8 weeks of gestation. Each calf was fed colostrum from its dam the first 3 days, whole milk from the college

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herd from the fourth day to the fourth week inclusive, and separated milk thereafter. Colostrum, whole milk, and separated milk were fed at the rate of 10 per cent of the body weight of the calf per day to a maximum of 12 lb. After the calves were 2 weeks of age, a concentrate mixture and alfalfa hay were fed *ad libitum*. Several hours daily the calves were released into an open pen. Additional details of feeding and management have been recorded (25).

Collection, preparation, and analysis of blood. Collections of samples of venous blood were made before colostrum consumption, daily the first 3 days, weekly from 1 to 6 weeks of age, and biweekly thereafter to 10 weeks of age. Analysis for the various constituents in the whole blood and the serum was continued either throughout the experiment or to the period when a uniform level apparently had been reached (tables 1 and 2).

Cell volume (hematocrit values), hemoglobin, and the minerals, calcium, magnesium, sodium, and potassium, were determined on samples of whole blood. The serum analysis included all the foregoing minerals and inorganic phosphorus in addition. The procedures used are outlined in figure 1. Where either blood or serum and one or more other substances are listed together, it is implied that they were mixed thoroughly.

The hematocrit readings reported herein are uncorrected for cell volume; therefore the values are considered as apparent rather than as absolute. It is recognized that when salts, such as sodium oxalate, are used as anticoagulants, there is a tendency for the individual cells to shrink (23), thus reducing the mass of the corpuscles. Contrariwise, the unavoidable entanglement of considerable plasma among the compacted cells tends to increase the apparent volume (9). Since these opposing tendencies are not entirely compensatory, the resulting volume is abnormally high. Thus calculations based on the apparent corpuscular-plasma ratio must be regarded as approximations suitable for comparative purposes only.

The only modifications in addition to those indicated in figure 1 were as follows:

Hemoglobin. This was determined by the Sanford and Sheard (18) method using the Evelyn photometer at 540 mμ to ascertain the intensity of color in the final solution. The Evelyn photometer was standardized by direct comparisons with a Bausch and Lomb spectrophotometer previously calibrated at 542 mμ (19).

Potassium. In this procedure (12), final concentrations were measured colorimetrically by means of the Evelyn photometer using a 440 mμ filter.

Inorganic phosphorus. This component of serum was determined by the procedure of Fiske and Subbarow (6), except that the final readings were made on the Evelyn photometer using a 620 mμ filter instead of the 660 mμ usually recommended.

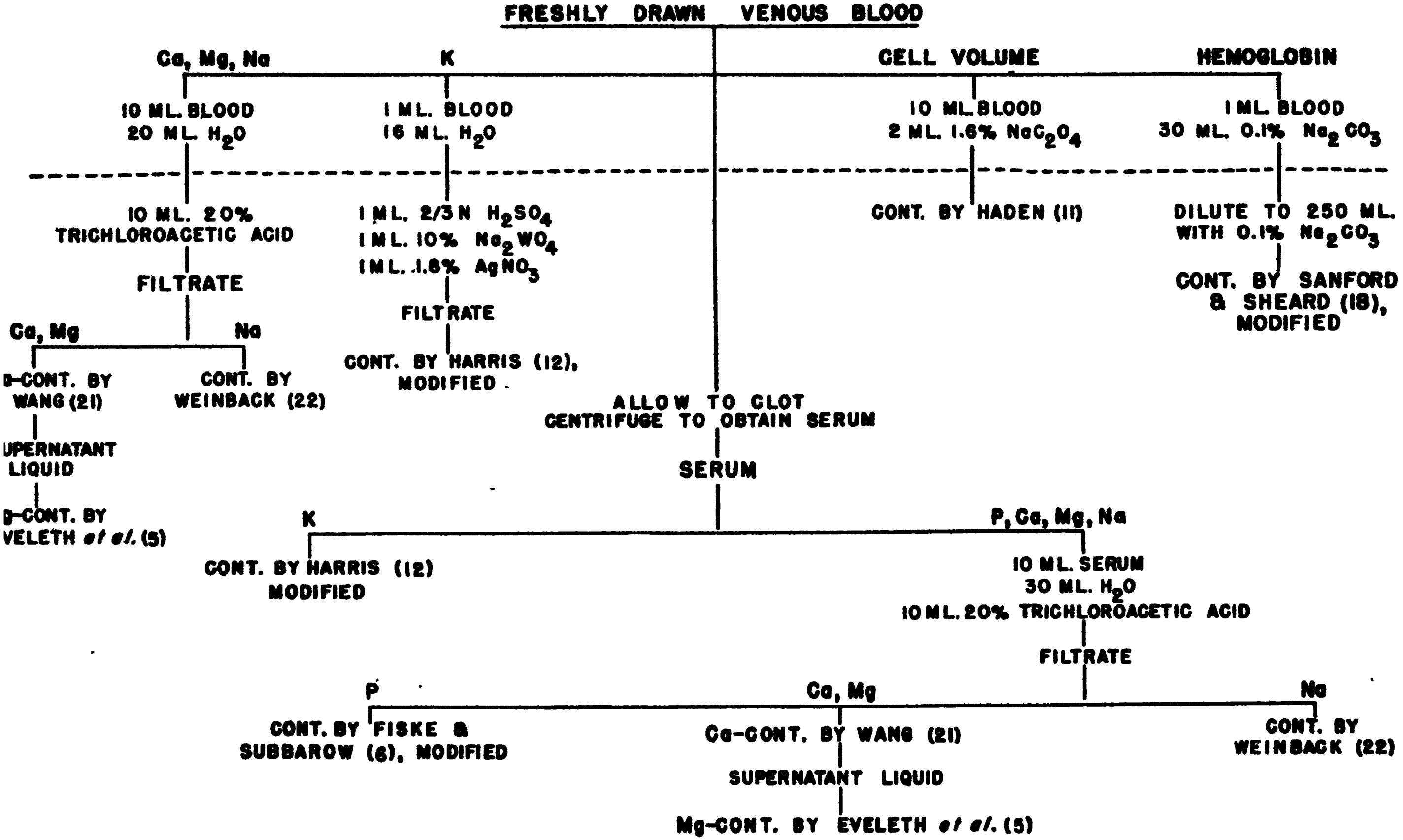

FIG. 1. Procedures followed in the preparation and the analysis of blood and of serum. (Steps indicated above broken line wer rformed immediately after samples were collected and those below, after the samples were transferred to the laboratory.)

RESULTS

A preliminary survey of the analytical data revealed that the variations among individuals within each breed exceeded the variability among breeds. Therefore, the data from all 11 calves were included in a single average for each stage of development at which determinations were made.

Blood serum. Means and standard errors for inorganic phosphorus, calcium, magnesium, sodium, and potassium of blood serum are shown in table 1. Of these elements, inorganic phosphorous exhibited the most pronounced change; it increased from birth to 3 weeks of age, the percentage increases being 13.4 by the third day and 26.9 by the third week. The calcium concentration was uniform during the period of colostrum ingestion but subsequently slowly decreased to 5 weeks of age, the degree of reduction being 13.8 per cent. The resulting ratios of calcium to inorganic phosphorus decreased from 1.8:1 at birth to 1.3:1 at 4 weeks. Though the levels of magnesium fluctuated from period to period, no definite trends were revealed; the ratios of calcium to magnesium, ranging from 5.1:1 to 4.2:1, thus tended to decrease as the calcium values declined. Sodium values decreased slightly (9.9 per cent) during the first 2 weeks, after which the levels tended to be uniform. Except for a slight reduction during the first 2 weeks, the potassium values manifested little variation; hence the range in sodium-potassium ratios was relatively small, 16.7:1 to 14.6:1.

TABLE 1

Average concentrations of several inorganic constituents in the blood serum of 11 normal dairy calves

Age of calf	Concentration of minerals in blood serum				
	Inorganic phosphorus	Calcium	Magnesium	Sodium	Potassium
(days)	*(mg./100 ml.)*	*(mg./100 ml.)*	*(mg./100 ml.)*	*(mg./100 ml.)*	*(mg./100 ml.)*
0	6.7 ± 0.29	12.3 ± 0.23	2.4 ± 0.10	384 ± 8.0	23 ± 0.7
1	6.9 ± 0.26	12.3 ± 0.19	2.6 ± 0.05	372 ± 9.4	25 ± 0.8
2	7.6 ± 0.15	12.3 ± 0.26	2.4 ± 0.06	372 ± 8.6	24 ± 0.5
3	7.6 ± 0.17	11.9 ± 0.19	2.4 ± 0.08	369 ± 10.2	23 ± 0.5
7	8.1 ± 0.20	11.2 ± 0.14	2.5 ± 0.04	352 ± 8.6	24 ± 0.3
14	8.3 ± 0.25	11.1 ± 0.13	2.4 ± 0.05	346 ± 6.0	22 ± 0.3
21	8.5 ± 0.18	11.0 ± 0.15	2.6 ± 0.06	356 ± 8.3	22 ± 0.6
28	8.5 ± 0.23	10.9 ± 0.14	2.5 ± 0.04	362 ± 7.3	22 ± 0.4
35	8.2 ± 0.32	10.6 ± 0.13	2.4 ± 0.06	352 ± 5.2	22 ± 0.5
42	8.1 ± 0.23	10.7 ± 0.18	2.4 ± 0.04	350 ± 6.0	21 ± 0.3
56	...	...	...	...	22 ± 0.4
70	...	...	...	...	22 ± 0.6

Whole blood. The means, including their standard errors, for various constituents of whole blood are presented in table 2. Cell volume (hematocrit values) and hemoglobin decreased 31.0 per cent and 29.8 per cent, respectively, during the first 3 weeks after birth, the rate of decline being more rapid during the first 3 days than later. After the third week, both constituents exhibited a gradual upward trend that continued during the remainder of the experimental period. Sodium values of the blood decreased 10.4 per cent during the first 2 weeks. After the period of decline, the concentrations gradually returned to approximately the initial level. The potassium content of the blood immediately after birth was almost three times as high as that at 10 weeks of age. With the exception of the accelerated reduction during the first 3 days, the rate of decrease tended to be uniform to the eighth week, at which time the values appeared to level off. The concentrations of calcium and of magnesium varied from period to period but manifested no definite trends that could be associated with changes in either age of calf or feeds consumed.

TABLE 2

Average levels of cell volume, hemoglobin, and several mineral constituents of the whole blood from 11 normal dairy calves

Age of calf	Levels of constituents in whole blood					
	Cell volume*	Hemoglobin	Calcium	Magnesium	Sodium	Potassium
(days)	*(%)*	*(g./100 ml.)*	*(mg./100 ml.)*	*(mg./100 ml.)*	*(mg./100 ml.)*	*(mg./100 ml.)*
0	42 ± 1.8	12.4 ± 0.66	9.0 ± 0.27	2.6 ± 0.08	308 ± 13.9	141 ± 4.4
1	39 ± 1.5	12.0 ± 0.53	9.2 ± 0.24	2.8 ± 0.06	291 ± 11.6	134 ± 4.8
2	37 ± 1.6	11.3 ± 0.55	9.3 ± 0.24	2.6 ± 0.05	292 ± 9.7	125 ± 4.7
3	34 ± 1.8	10.7 ± 0.51	9.2 ± 0.22	2.7 ± 0.08	290 ± 13.3	119 ± 4.4
7	33 ± 1.9	10.2 ± 0.57	8.8 ± 0.17	2.7 ± 0.04	278 ± 12.0	111 ± 3.2
14	30 ± 2.0	9.3 ± 0.54	8.9 ± 0.11	2.6 ± 0.05	276 ± 10.6	94 ± 3.7
21	29 ± 1.9	8.7 ± 0.56	9.4 ± 0.23	2.7 ± 0.06	294 ± 10.4	82 ± 3.3
28	31 ± 1.7	9.0 ± 0.54	9.0 ± 0.19	2.7 ± 0.05	298 ± 4.5	80 ± 3.6
35	30 ± 1.1	9.1 ± 0.35	8.5 ± 0.10	2.6 ± 0.05	313 ± 8.1	69 ± 4.1
42	30 ± 0.8	9.2 ± 0.25	8.6 ± 0.14	2.5 ± 0.04	311 ± 7.4	62 ± 2.3
56	32 ± 0.9	9.7 ± 0.30				54 ± 1.9
70	33 ± 1.2	10.7 ± 0.39				53 ± 1.4

* Hematocrit values.

A comparison of the data in table 1 with those of table 2 reveals higher values for calcium and sodium in blood serum than in whole blood; whereas the reverse was true of the levels for magnesium and potassium. Thus it is apparent that calcium and sodium were concentrated primarily in the serum phase and magnesium and potassium in the cellular components of whole blood.

In contrast to the small changes in the proportion of sodium to potassium in the serum of the calves, the sodium-potassium ratio in the whole blood gradually increased from 2.2:1 at birth to 5.0:1 at 6 weeks.

The calculated ratio of sodium to potassium in the cellular fractions, based on hematocrit values, suggested that the proportions changed as the calves developed. During the first 4 weeks following birth, the potassium content of the cells was greater than the sodium; thereafter the sodium concentration was higher. This change in relationship was due not only to the reduction in potassium but also to the increase in sodium.

Computations of the approximate distribution of potassium between the serum and the cells (based on hematocrit readings) in the whole blood at different periods indicated that at birth only 10 per cent of the total was in serum, but at 10 weeks of age the percentage had increased to 28. The sodium distribution varied slightly, but the fluctuations were not of sufficient magnitude to be considered significant.

DISCUSSION

The complexity of the interaction of the mineral elements in the body functions renders interpretation of the changes in concentration of individual constituents of the blood difficult. The concomitant reduction in the apparent cell volume and the hemoglobin of the blood of the calves during the first 3 weeks of postnatal life raises a question concerning the physiological causes and significance of the trends. Though the large amounts of blood withdrawn from the young calves during the first 3 days probably accentuated the rate of decrease during this period, the general downward trends are in accord with the observations of Fraser (7), who found that the hemoglobin concentration and the number of red blood cells in calves and lambs decreased during the first week following birth. Occasionally, however, low hemoglobin values have been noted (24) in the newborn calf. The levels in these cases either remained approximately uniform or increased slightly. Apparently the trends of hemoglobin during the first 2 or 3 weeks of postnatal life are influenced by the initial level.

According to a review by Davidson and Leitch (2), a high hemoglobin level in mammals at birth is regarded as an adaptation to relatively inefficient aeration *in utero*. With the establishment of lung breathing, there is believed to be a surplus of hemoglobin; hence red blood cells are destroyed, a so-called "physiological destruction". It was suggested further that this alteration is a process of replacement of fetal erythrocytes by cells of a different type. This physiological reduction is believed to be independent of iron reserves and dietary intake. The changes in the hematocrit values and hemoglobin levels in the blood of the young calves observed in this study suggest the occurrence of "physiological anemia", particularly in the early stages of decline. Whether the apparent activation of erythropoiesis when the calves were about 4 weeks of age was stimulated by the stage of growth (2) or by the preceding dietary changes remains to be determined. Further hematological studies of calf blood to determine the early postnatal changes in types of cells and additional nutritional investigations to ascertain the responsiveness of the hemoglobin content of the blood to dietary supplements are indicated.

One of the most striking characteristics noted in the blood of the young calf is the high potassium content at birth followed by a gradual reduction over a period of 8 to 10 weeks to the normal adult level (10). Another investigator (9) also has observed this phenomenon, but its physiological significance is obscure.

As previously indicated, potassium is found primarily in the red blood cell; therefore changes in the cell volume (hematocrit) would be expected to affect the concentration of potassium in the whole blood. Since the downward trend of potassium continued after the hematocrit and the hemoglobin values began increasing, factors other than corpuscular volume *per se* apparently were involved. If the suggestion (2) that the type of cells change during the early postnatal development applies to calves, it is conceivable that the high potassium content is characteristic of the fetal erythrocytes. On the basis of this conjecture, changes in the concentration of potassium of blood suggest that most of the displacement of the fetal red cells had occurred by the eighth to the tenth week following birth. Though reduction may be due to excessive egression from the cells, the constancy of potassium in the serum tends to invalidate this possibility.

The metabolic significance of the high potassium values in the neonatal calf is subject to speculation. Shohl (20, p. 124) stated that when growth is rapid, the need for potassium increases. Miller's investigations (14) with rats indicated that a deficiency of this element during early development not only may prevent growth but also may cause physiological disturbances that are manifested at a later age, even though the potassium supply is raised to adequate levels. If similar responses occur in the bovine, the high level of potassium in the blood of the neonatal calf possibly may be insurance against the development of subsequent anomalies. This postulation is in harmony with the fact that colostrum, the first food of the calf, is considered to be low in potassium (8). However, the normal dietary changes to which the calves were subjected did not alter perceptibly the downward trend in the potassium concentration in the blood. The extent to which this reduction would be amenable to supplemental feeding of potassium remains to be determined.

In the calf, as in other species, sodium of the blood occurs principally in the serum. The amount in the red blood cells of the human subject is so small that its presence has been questioned (20, p. 121), but this is not true of other species, including cattle. Kerr (13) reported that in the dog, the sheep, and the cow sodium predominated over potassium in the red blood cells. The data presented herein indicated that in the early postnatal stages of the calf there was a preponderance of potassium in the cellular fraction, but after 4 weeks of age the computed sodium content of the cells was greater than the potassium, a relationship similar to that reported for the cow (13).

According to Bunge's thesis, as reviewed by Shohl (20, p. 123), an excess intake of either potassium or sodium causes excretion of the other. The dietary sodium-potassium ratio apparently was not a major factor contributing to the ratio of these two elements in the blood of young calves observed in this study. Moreover, there was not a reciprocal relationship in the concentrations of sodium and potassium of the blood as reported by Groenewald (10), who found that the reduction in potassium concentration was correlated with an increase in sodium.

The responses of sodium concetrations in the whole blood and in the serum to dietary changes are indefinite. Though the increases followed the addition of hay and concentrates (containing 1 per cent supplemental sodium chloride) to the diet, the changes, particularly in the serum, were not of sufficient magnitude to be considered significant.

The calcium in blood, according to Shohl (20, p. 133), is found wholly or almost wholly in the serum, the red cell membrane being impermeable to this element. Gradual reduction of serum calcium of the calf following the early postnatal stages corroborates the findings of other investigators (1, 9, 10). Computations based on the corpuscular-plasma volumes indicated the presence of considerable calcium in the cellular fraction of blood also. The validity of these calculations is questionable in view of the inadequacy of hematocrit measurements as indicators of the absolute red cell volume.

The inorganic phosphorus of the serum increased considerably during the early stages of postnatal development. Studies (9, 10, 15) of the inorganic phosphorus of the whole blood, collected at frequent intervals during early postnatal life, revealed trends similar to those noted herein for serum. Since inorganic phosphorus is concentrated almost entirely in the serum (10), the increases in the whole blood are indicative of the changes in concentration of this phosphorus fraction in the serum.

Unlike calcium and inorganic phosphorus, magnesium is present in slightly greater concentration in the whole blood than in the serum of calves. This is in accord with the results from other studies (9) of calf blood, but contrary to the findings from investigations (4) of the compositions of blood from older cattle. There is no obvious explanation for this difference.

Sodium, potassium, calcium and magnesium ions in the body fluids must be balanced in order that irritability and contractility may be produced (20, p. 118). Though the concentrations and the distributions of these elements in the serum and the corpuscular phases of the whole blood varied with advancement in the age of the calf, the metabolic significance of these changes is obscure.

Transitions in the typical dietary regime did not alter the concentrations of the mineral constituents of calf blood and blood serum so perceptibly as they did the cartenoid and vitamin A values (25). It is possible that such factors as solar irradiation during the period of experimentation (summer and fall), water consumption, and ingestion of foreign matter from the barnyard might have modified the effects of the diet. Determination of the responses of the minerals of the blood of young calves to various dietary supplements warrants further exploration.

SUMMARY

During the first 6 to 10 weeks of postnatal development of 11 normal calves, determination of hematocrit values and of hemoglobin levels in the blood and of the concentrations of several inorganic constituents in the blood and in the serum were made.

Hematocrit values and hemoglobin concentrations declined during the first 3 weeks after birth but gradually increased during the following 7 weeks.

Inorganic phosphorus of the blood serum increased from 6.7 mg./100 ml. at birth to 8.5 mg./100 ml. at 3 weeks, remaining relatively constant thereafter.

Calcium of the whole blood ranged from 8.5 mg./100 ml. to 9.4 mg./100 ml. but revealed no consistent trends, whereas in serum the level decreased slightly but steadily from 12.3 mg./100 ml. during the period of colostrum consumption to 10.6 mg./100 ml. at 5 weeks of age.

Magnesium of whole blood varied from 2.5 mg./100 ml. to 2.8 mg./100 ml., and of serum from 2.4 mg./100 ml. to 2.6 mg./100 ml., but the concentration in neither exhibited a definite trend as the calves developed.

Concentrations of sodium in the whole blood decreased slightly the first 2 weeks but increased to the initial level within the following 3 weeks, the extremes being 276 mg./100 ml. and 313 mg./100 ml.; similar general trends were noted in the serum in which the range in concentrations was 346 mg./100 ml. to 384 mg./100 ml.

Potassium of the whole blood declined steadily from an average of 141 mg./100 ml. at birth to an average of 53 mg./100 ml., the level in adult cattle, at 10 weeks; the concentrations in the serum exhibited little variation, ranging from 21 mg./100 ml. to 25 mg./100 ml.

A comparison of the levels of minerals in whole blood and in serum indicated that the concentrations of sodium and calcium were greater in serum than in the cells; whereas the reverse was true of potassium and magnesium.

Computations, based on hematocrit values, indicated that the amount of potassium in the cells predominated over that of sodium during the first 4 weeks of postnatal life; subsequently sodium concentrations predominated over potassium in the cellular fraction.

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THE KEEPING QUALITY OF DRY WHOLE MILK SPRAY DRIED IN AN ATMOSPHERE OF AN INERT GAS[1,2]

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The drying of milk in an atmosphere of an inert gas such as nitrogen or carbon dioxide in order to eliminate or retard oxidation of the lipids has been suggested frequently. Ekenberg (4) in 1911 was granted a U. S. patent for a process for spray drying milk in an atmosphere of carbon dioxide and water vapor. Dickinson and Moreton (3) received a U. S. patent in 1943 on a method involving spray drying in carbon dioxide or "any other oxygen-free gas". A French patent was granted in 1921 to Vitoux and Porcher (7) for a drier utilizing an inert gas.

Obviously, to use a gas other than air would be practical only if the gas was very cheap or could be dehumidified for reuse. Exhaust flue gases might be satisfactory if combustion was so regulated as to obtain the desired low oxygen level in the gases and yet sufficiently complete to eliminate objectionable characteristics such as carbon specks.

The 1907 patent of Merrell *et al.* (5) on spray drying provided for possible recirculation of the air. An arrangement of cooling coils was used to partially dehumidify the air. Ekenberg (4) utilized the same principle. Bichowsky (1), in a patent granted in 1941, provided for recirculation of air in tray drying by regulating the humidity of the air through contact with a hygroscopic solution of controlled temperature and humidity.

A spray drier[3] which permits recirculation of the drying fluid (air, nitrogen, or carbon dioxide) has been in successful operation in the laboratories of the Dairy Division of the University of Minnesota since October, 1943. Dehumidification is accomplished in this drier by passing the exhaust gas from the drier counter-current through sprays of water. In a later design, a standard finned-type air conditioning unit was used.

There is little advantage in cooling the gas below 70° F. (21.1° C.). The cooled gas is, of course, approximately saturated with water vapor at the temperature to which it is cooled, but at 70° F. only 0.01578 lb. (6) of water vapor is required to saturate 1 lb. of air. This amount is less than the water vapor usually carried by the air used in conventional driers, particularly during the summer months.

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[2] Aided by a grant from the Minnesota Research Foundation.

[3] Construction of the drier was supervised by Mr. J. A. Phelan, Central Scientific Shops, University of Minnesota.

Assuming sufficient cooling surface so that there can be a 10° F. (5.5° C.) temperature rise in the cooling water, about 1 gallon of water is required per pound of gas (nitrogen) circulated. This figure is based on nitrogen 15 per cent saturated at 170° F. (77° C.), which is about normal for spray drier operation and will vary somewhat depending upon the temperature and humidity of the exhaust gas from the drier. Under these conditions, about 19 gallons of cooling water are required per pound of drying effect or 28.5 gallons per pound of powder dried from condensed milk with a solids content of 40 per cent.

DESCRIPTION OF DRIER

Schematic diagrams of the drier are shown in figures 1 to 3. The principal features are:

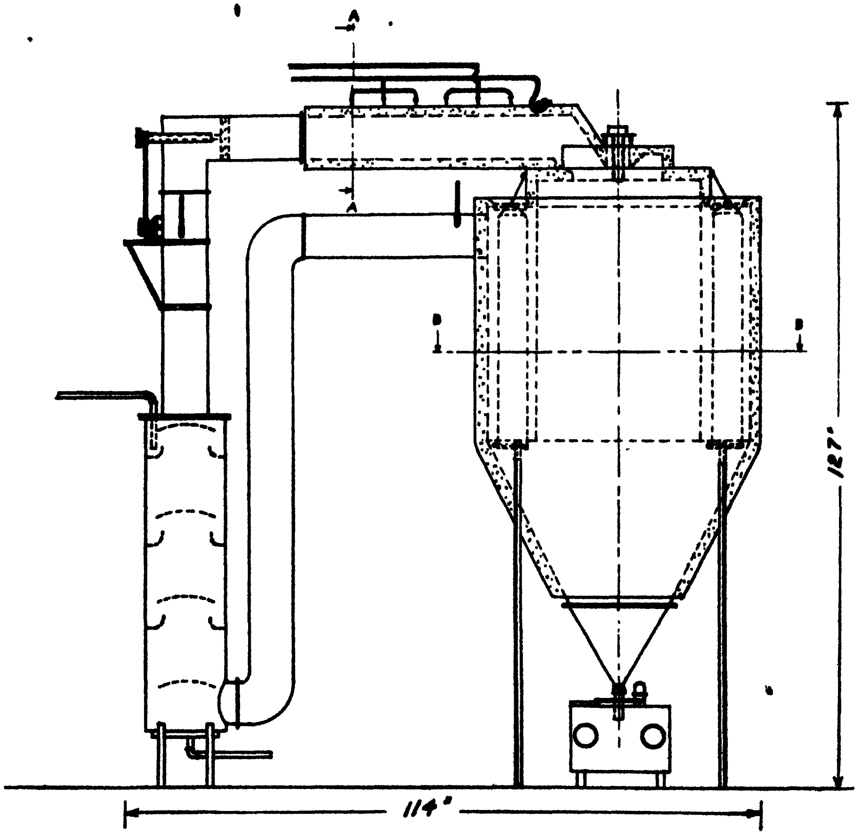

FIG. 1. Schematic diagram of drier.

I. The heater and fan.

Five 1,400-watt chromalox "Koilfin" heaters manufactured by the Edwin L. Weigand Company, 7500 Thomas Boulevard, Pittsburgh, Pa., are used for heating. A circulation of up to 120 cubic feet per minute of gas is provided by a two-blade fan driven through variable speed pulleys by a 0.25 h.p. electric motor. Three of the heaters can be thrown in or out of the circuit by individual snap switches. The other two heaters are regulated thermostatically.

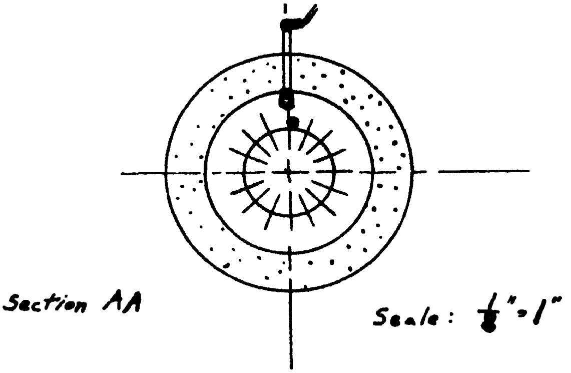

FIG. 2. Cross section of heater section.

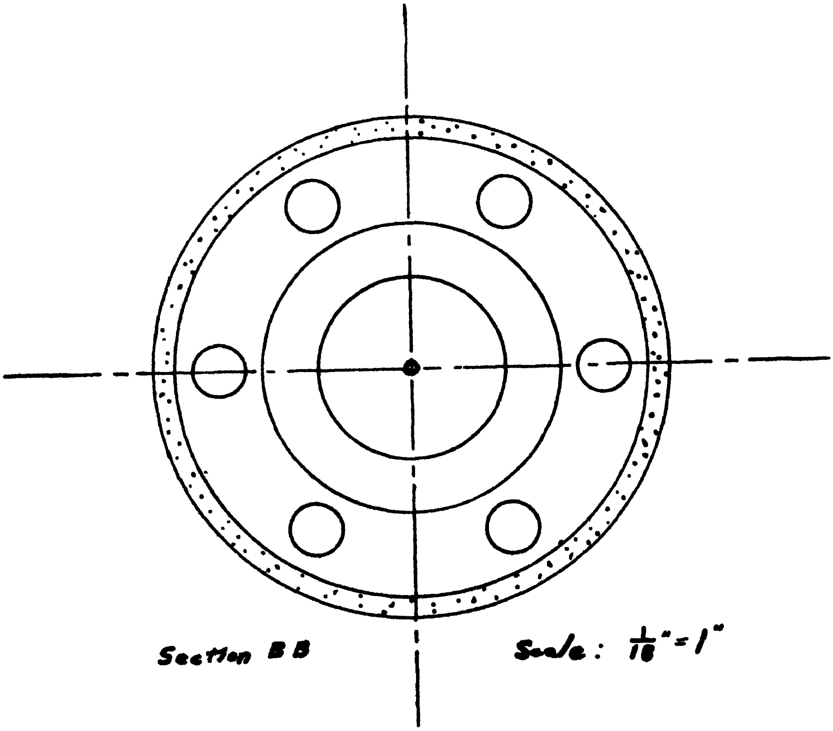

FIG. 3. Cross section of drier chamber.

II. The atomizer.

A diagram of the two-fluid stainless steel atomizer is shown in figure 4. The bore of the milk tube is 4 mm. tapering to 1 mm. at the tip, which has an outside diameter of 1.5 mm. The atomizing gas (air, nitrogen, or carbon dioxide) at a pressure of 25 to 70 lb. per square inch emits around the tip of the milk tube through a 2 mm. orifice in the cap. The rate of milk flow can be varied by regulating the pressure on the milk line. Compressed air, nitrogen, or other gas introduced above the milk in a closed container is used as the source of pressure. A relief valve is installed in the container. The rate of flow is determined by noting the loss in weight of the milk and container placed on a dial scale of suitable sensitivity.

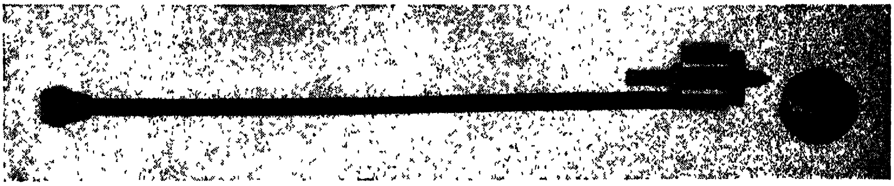

FIG. 4. Two-fluid atomizer for drier.

III. The drying chamber.

The atomizer is mounted so that the milk spray enters the gas stream at a point level with the top of the drying chamber. The dry milk falls to the bottom of the cone and thence to the box below the cone, where it may be canned without contact with the air. The cans and sealer may be manipulated through rubber gloves attached to flanged holes in the side of the box.

IV. Powder removal from air stream.

The gas leaving the cone passes upward through six filter bags evenly spaced around the drying chamber between the inner cylinder and the outer wall. The bags are standard bags for a Roger's drier shortened to the required length. The rods to which the upper ends of the bags are attached are hooked to supports outside of the drying chamber so that the bags may be shaken when necessary. A reasonably gas-tight seal is provided by sleeves of flexible rubberized cloth clamped at one end to the rods and at the other to the rims of 6-inch openings in the top of the chamber.

V. Dehumidification of air.

The exhaust air duct conveys the gas to the bottom of the "condenser". Water at about 50° F. (10° C.) is fed into the top of the chamber and falls over a series of baffles arranged so that the air must pass counter-current to the water flow through a series of water sprays. A water seal in the overflow pipe from the bottom of the chamber prevents entrance of air during operation.

OPERATION OF DRIER

The drier was designed to remove at the maximum about 10 lb. of water per hour. Operating on 40 per cent condensed whole milk, a capacity of 5 lb. of dry whole milk per hour easily can be attained. Best results have been secured with the temperature of the inlet gas at about 300° F. (149° C.) and that of the exhaust gas at 160° F. (71° C.). Dry milk with a moisture content not higher than 2.0 per cent and yet with a solubility index not greater than 0.1 ml. easily can be obtained under these conditions.

The drier operates equally well on air, nitrogen, or carbon dioxide. The system may be charged with nitrogen or carbon dioxide by alternately admitting gas from a tank to build up pressure equivalent to about 1 inch of water and then drawing an equivalent vacuum through an outlet in the box below the cone. Gas for analysis can be taken from the same outlet. Using this procedure it is possible to reduce the oxygen content of the gas in the system to below 2 per cent with one 13-lb. tank of nitrogen. Using nitrogen as the atomizing gas, the oxygen content of the gas in the system drops during operation to below 1 per cent in less than 1 hour and to below 0.5 per cent in 2 hours. One tank of nitrogen will furnish gas at 70 lb. pressure to the atomizer for about 2 hours. The oxygen content of the gas in the head space of the canned dry milk is comparable to that of the gas in the chamber at the time of drying.

This drier, as constructed and operated, is, of course, strictly an experimental unit. It is believed, however, that the general scheme could be used commercially without prohibitively increasing the cost of the dried product.

Present commercial driers probably could not be adapted readily to the use of an inert gas because of their large volumes in relation to drying capacities and the difficulties of charging the systems with nitrogen.

KEEPING QUALITY OF DRY WHOLE MILK

Eight comparisons have been made in which split lots of the same milk have been dried, one in nitrogen and the other in air. The milk was high quality mixed milk from the University herd and was preheated to 165° F. for 30 minutes and condensed in stainless steel equipment to about 40 per cent total solids before drying. The milk dried in nitrogen was canned in the atmosphere in which it was dried. That dried in air was gas-packed to equivalent oxygen levels. Multiple gassing (2), with 24-hour intervals between gassings to permit diffusion of oxyen from the air cells, was necessary to obtain the lower oxygen levels.

Every effort was made to control the moisture content of the powder to within the range of 2.0 to 2.5 per cent, but this was not attained in all instances. Samples of the canned powder were stored at 100° F. and graded at intervals during a period of up to 22 months. The milk was reconstituted for scoring with distilled water at 70° F. and was examined within an hour. Scores were placed by a panel of never less than five and usually six persons selected from among a larger group because of their ability to distinguish among flavors. The student's score card for fluid milk, in which the normal range in flavor score is 25 to 40, was used. Reconstituted milk prepared from freshly dried milk usually is scored 37 with the criticism "cooked" by the panel.

Data on all of the first five lots will not be reported in detail since with two of these lots the moisture content of the nitrogen-dried powder was excessive and with one lot the desired oxygen level was not attained. Summaries of the data for five of the lots are shown in tables 1 to 5.

The keeping quality of the powder appears to have been influenced by the differences in the amount of oxygen in the can and not by whether the powder was dried in nitrogen or in air. An interesting observation, as shown in lots 1 and 2, was that powder dried in nitrogen and then removed

TABLE 1

Comparison of keeping qualities of powdered whole milk spray dried in nitrogen and in air

(Summary of data for lot 1)

Dried in	Remarks	7-day oxygen level	Scores after storage at 100° F. for	
			5 mo.	10 mo.
		(%)		
Nitrogen	Canned in air	17.0	Tallowy	...
Nitrogen	1st half run	3.0–4.0	.....	32.5
Nitrogen	2nd half run	2.0–3.0	35	34
Air	Canned in air	20.8	Tallowy	..
Air	Single gassed	2.0	35	34

TABLE 2

Comparison of keeping qualities of powdered whole milk spray dried in nitrogen and in air

(Summary of data for lot 2)

Dried in	Remarks	7-day oxygen level	Scores after storage at 100° F. for			
			Initial	3 mo.	8 mo.	15 mo.
		(%)				
Nitrogen	....	1.4–3.0	37	34	34	31
Nitrogen	Canned in air	19.7	37	28	Tallowy	....
Air	Single gassed	1.5	37	35	35	32.5
Air	Canned in air	20.8	37	Tallowy	. .	...

TABLE 3

Comparison of keeping qualities of powdered whole milk spray dried in nitrogen and in air

(Summary of data for lot 6)

Dried in	Remarks	Oxygen level	Scores* after storage at 100° F. for			
			Initial	3 mo.	10 mo.	12 mo.
		(%)				
Nitrogen	1st half run	0.8–1.0	37	35.75	35.5	35.25
Nitrogen	2nd half run	0.4–0.8	37	35.50	36.25	35.25
Air	Double gassed	0.8	37	35.25	35.25	35.25

* Averages of scores on two cans of powder separately reconstituted.

TABLE 4

Comparison of keeping qualities of powdered whole milk spray dried in nitrogen and in air

(Summary of data for lot 7)

Dried in	Remarks	7-day oxygen level	Scores after storage at 100° F. for				
			Initial	4 mo.	12 mo.	16 mo.	22 mo.
		(%)					
Nitrogen	1st half run	1.50–2.00	37	35.75	36.25	34.75	34.75
Nitrogen	2nd half run	1.00–1.50	37	35.75	36.25	34.50	34.75
Air	Single gassed	2.00	37	35.50	34.25	33.5	......
Air	Double gassed	1.10	37	35.75	35.25	34.5	......

* Averages of scores on two cans of powder separately reconstituted.

TABLE 5

Comparison of keeping qualities of powdered whole milk spray dried in nitrogen and in air

(Summary of data for lot 8)

Dried in	Remarks	7-day oxygen level	Scores after storage at 100° F. for				
			Initial	6 mo.	9 mo.	13 mo.	20 mo.
		(%)					
Nitrogen	1st half run	0.6–1.0	37*	35*	34.5*	35*	34.5*
Nitrogen	2nd half run	0.5–0.6	37	35.75*	35.5*	35.5*	35*
Air	Single gassed	1.7	37	34	35	33.5	33
Air	Double gassed	0.7	37	35	34.5	34.5	34
Air	Held under vacuum before gassing	0.4	37	35	35	35	35

* Averages of scores on two cans of powder separately reconstituted.

from the drier and canned in air actually "desorbed" nitrogen so that the 7-day oxygen level was materially lower than that of powder dried and canned in air.

The data show an improvement in the keeping quality of the powder with decrease in the oxygen level. At oxygen levels of 1 per cent or lower, the powder retained its quality remarkably well. Deterioration was most rapid during the first months, and after an initial drop in score the powder became virtually stable.

The gases in the cans were analyzed for oxygen and carbon dioxide at each examination period. Oxygen was not present in the gases in detectable amounts after 6 months storage. The carbon dioxide content increased gradually throughout the storage period. These powders were subjected to various other analyses, but since much more extensive data obtained in studies on the effects of oxygen and moisture levels on the keeping quality of dry whole milk now are being prepared for publication, they will not be reported at this time.

SUMMARY

The drying of whole milk or other oxygen-labile materials in an atmosphere of nitrogen or carbon dioxide has been shown to be entirely practical. Drying of the gas to permit re-use can be affected by cooling at an expenditure of not more than 20 gallons of 50° F. water per lb. of water removed. The amount of gas required would be excessive with the conventional spray driers now available.

The keeping quality of dry whole milk is influenced materially by the amount of oxygen left in the container, but not by whether the milk is dried in inert gas or in air. At oxygen levels of 1 per cent or below, dry whole milk of low moisture content deteriorates very slowly, even at 100° F.

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TOTAL DIGESTIBLE NUTRIENTS AND PROTEIN LEVELS FOR DAIRY BULLS USED IN ARTIFICIAL BREEDING

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There are few objective data available concerning the levels of total digestible nutrients (T.D.N.) and protein necessary to maintain the body weight of mature bulls (19). With the exception of the Russian work (11, 12, 20, 21), which the authors are in no position to evaluate, having seen only the abstracts, a review of the literature reveals little objective information concerning the effects of quantity and quality of rations on semen production (5) and fertility of mature dairy bulls. Most of the studies in this country have been with young bulls (7, 8). The increasing number and size of bull studs supplying semen from proved bulls for artificial insemination has focused more and more attention on the need for reliable information on feding the mature sire.

Many authors (3, 4, 10, 13) have recommended that dairy bulls be kept in vigorous physical condition but not too fat since high finish is believed to make them sluggish. Others (6, 11) have recommended that bulls gain in weight when in active service. The actual basis for their recommendations is not given.

Recognizing the situation just described, a series of investigations were planned in this laboratory to determine the nutritive requirements of mature dairy sires used routinely for artificial insemination. The first of these experiments was published earlier (14). However, as that experiment progressed, it became evident that more fundamental information was needed on the T.D.N. and protein requirements before other nutrient requirements could be studied adequately. Thus, the present paper deals with the results of an investigation to determine the effects of feeding controlled levels of T.D.N. and protein on semen production, fertility level, and liveweight changes of mature dairy bulls used routinely in artificial insemination.

EXPERIMENTAL METHODS AND MATERIALS

Since the establishment of the bull stud by the New York Artificial Breeders' Cooperative, Inc., the general feeding practices have resulted in T.D.N. intakes of approximately 120 per cent of recommended allowances for maintenance of dry dairy cows of equivalent weight (10). The rations generally fed consisted of concentrate mixtures containing approximately

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14 per cent total protein and mixed hay containing less than 50 per cent legumes. These feeds, at the levels fed, apparently had been satisfactory for the production of semen of high quality, the maintenance of fertility, and the maintenance of body weights. It seemed desirable to study levels at considerable ranges from the average in use. Therefore, the levels of 100, 120, and 140 per cent of recommended dry dairy cow maintenance requirements for T.D.N. (10) were chosen for study.

The basic formula for calculation of these T.D.N. allowances was 7.93 lb. T.D.N. $\times \frac{(\text{body weight})^{0.87}}{1{,}000}$. For the two higher levels, the allowance as determined for the 100 per cent level[2] was increased by 20 and 40 per cent, respectively. Since it was desired to use concentrate mixtures made up of varying proportions of the same feeds, the lowest level of protein chosen was 12 per cent. From some of the work mentioned above (5, 11, 12) it appeared that additional protein might be advantageous. Therefore, three levels of protein in the concentrate mixtures, 12, 16, and 20 per cent, were used for comparison.

Feeds used and management of the bulls. The ingredients used to make up each of the concentrate mixtures, chemical analysis of each mixture and that of the hay fed are given in table 1.

The experimental feeding was planned so that 60 per cent of the T.D.N. requirement of each bull was supplied by the hay fed and 40 per cent by the concentrate mixture fed. While this proportion of feeds has been found satisfactory for maintaining tidy middles on bulls, enabling them to serve readily, it is not known that it represents the ideal combination.

The amount of feed to be fed each bull during each experimental period, in order to meet the T.D.N. levels specified for each period, was established on the basis of the initial body weight of each bull. The resulting feeding schedule was not altered as a consequence of subsequent changes in body weight.

All bulls were exercised daily, either in a small dry lot or on a mechanical exerciser. The latter exercise was equivalent to a slow walk for approximately one mile. Throughout the experiment each bull was used for service approximately once every 12 days, at which time two or three ejaculates were collected.

The experimental design. For the study of the three T.D.N. levels and in order to remove statistically their possible carry-over effects, a "double change over" design, as described by Cochran *et al.* (2), involving three 90-day periods, was employed. Superimposed upon their design was a 270-

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[2] The 100 per cent level of recommended allowances of T.D.N. is the same as that given by Morrison (10) for "Good cows under usual conditions" for body weights up to 1,800 lb.

day continuous-type experiment for studying the three protein levels. Nine Holstein-Friesian and nine Guernsey bulls, averaging 6.3 ± 0.5[3] years of age and 1749 ± 79[3] lb. in body weight, were selected from the stud of the New York Artificial Breeders' Cooperative, Inc., on the basis of the likelihood that they would remain in active service during the projected 270-day experiment. Within each breed the bulls were divided into three groups containing three similar individuals. Assignment to groups was made on the basis of the information available concerning each bull's semen production, fertility, and body weight. The three groups within each breed then were assigned at random to the three protein levels. Within protein levels, the assignment of sequences of T.D.N. levels to each bull was done in a manner described by Cochran *et al.* (2). The completed design is shown in detail in table 2.

TABLE 1

Ingredients and chemical composition of the concentrate mixtures and hay fed

	Concentrate mixtures			
	12% total protein	16% total protein	20% total protein	Hay
Ingredients (lb.)				
Ground oats	780	680	585	50% timothy
Hominy feed	940	760	575	
Wheat bran	200	200	200	40% grasses
Linseed oilmeal	20	160	300	
Soybean oilmeal	20	160	300	10% clover
Steamed bone meal	20	20	20	
Salt	20	20	20	
Total	2000	2000	2000	
Chemical composition (%)				
Moisture	10.85	10.47	10.09	10.63
Crude protein	12.38	15.37	18.48*	8.88
Ether extract	3.90	3.83	4.26	1.64
Crude fiber	7.94	8.71	7.37	38.04
Nitrogen-free extract	59.66	56.42	54.93	34.71
Ash	5.27	5.19	4.87	6.10
Total	100.00	99.99	100.00	100.00
Total digestible nutrients (%)	76.55	76.54	76.49	49.20

* Because of one low value for crude protein during one period, the average of the chemical analyses of the theoretical 20% protein concentrate was somewhat below the planned level.

Measurement of the results. Data were recorded routinely on the characteristics of each ejaculate of semen produced by each bull at each collection period, on the fertility of the semen used for insemination, and on the changes in body weights of the bulls. For the statistical analyses of the semen characteristics the data were grouped by experimental periods, the average value being used as a single observation. Significance of treatment differences was tested by analyses of variance (9, 22).

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[3] Standard error of the mean.

The following criteria were used to evaluate the semen samples: volume, per cent of motile spermatozoa (1), concentration (number of spermatozoa per mm.^{3}) (17), and the methylene blue reduction time (1). Each semen sample which met the enumerated specifications for use in artificial insemination was diluted with yolk-citrate diluent (18, 23) and shipped by the Cooperative to its affiliated local units throughout New York state for use in inseminating cows. Semen samples which did not meet the specifications for shipment were discarded. This fact in itself was used as an additional overall criterion of semen quality.

TABLE 2

Experimental design showing assignment of bulls to treatments

(Each period 90 days in length)

12% protein group

Period	T.D.N.—Group 1 (Holsteins)			Period	T.D.N.—Group 2 (Guernseys)		
	Bull				Bull		
	1	2	3		4	5	6
I	100*	120	140	I	100	120	140
II	120	140	100	II	140	100	120
III	140	100	120	III	120	140	100

16% protein group

Period	T.D.N.—Group 3 (Holsteins)			Period	T.D.N.—Group 4 (Guernseys)		
	Bull				Bull		
	7	8	9		10	11	12
I	100	120	140	I	100	120	140
II	120	140	100	II	140	100	120
III	140	100	120	III	120	140	100

20% protein group

Period	T.D.N.—Group 5 (Holsteins)			Period	T.D.N.—Group 6 (Guernseys)		
	Bull				Bull		
	13	14	15		16	17	18
I	100	120	140	I	100	120	140
II	120	140	100	II	140	100	120
III	140	100	120	III	120	140	100

* Figures within the T.D.N. groups represent the T.D.N. level fed in terms of % of Morrison's (10) recommended maintenance standard for good dry cows under usual conditions.

The dilution rates employed varied from 1:39 to 1:99 depending on the need for semen on any particular day. These varying dilution rates were not confounding factors within the experiment, as shown by the investigations of Salisbury (15).

The evidence on the fertility of the semen samples was obtained from the number of services to cows being bred for the first time in any service period and whether or not these cows returned to artificial service during a period of 5 months. For each experimental period the total number of these first services and the total number of 5 months' non-returns to these same first services were used to calculate the per cent of non-returns (16). The significance of differences observed was tested by covariance analysis (22).

In order to establish the initial liveweight and the body weight changes of each bull, the bulls were weighed on 3 consecutive days at the beginning of the experiment and at the end of each 90-day experimental period.

RESULTS AND DISCUSSION

The results of the investigation are presented in five parts, namely, the calculated average intake of digestible protein and T.D.N. of each group at each level of intake, the characteristics of the semen produced, the fertility of the semen used, the changes in body weights of the bulls, and a tentative feeding standard for mature dairy bulls.

Calculated intakes of T.D.N. and digestible protein. The intakes of T.D.N. and digestible protein for each of the groups of bulls at the three levels fed are shown in table 3. The values were calculated from the amounts of feed fed, the chemical composition of the feeds, and the average digestion coefficients given by Morrison (10).

TABLE 3

Calculated average total pounds T.D.N. and digestible protein fed per bull per day

Protein level of concentrate	Constituent	Calculated amounts at T.D.N. levels of			
		100%	120%	140%	Av.
(%)		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
12	T.D.N.	12.98	15.64	18.18	
	Digestible protein	1.25	1.51	1.75	1.51
16	T.D.N.	12.79	15.31	17.92	
	Digestible protein	1.40	1.68	1.96	1.68
20	T.D.N.	12.79	15.35	17.74	. .
	Digestible protein	1.64	1.97	2.28	1.96
Av. % T.D.N.		12.85	15.43	17.95	...

Semen characteristics. The mean values and the least differences between means required for statistical significance on ejaculates from which the means were derived are shown in table 4. These means represent first, second, third, and in few instances, fourth ejaculates.

It should be pointed out that the effect of protein levels was measured less precisely by the experimental design used than was the effect of the T.D.N. levels. The results as shown in table 4 are interpreted to mean that rather wide ranges of T.D.N. accompanied by considerable shifts in body weight, as will be shown later, can be fed mature bulls for at least 90 days without influencing semen production. The data also indicate that no more protein need be fed than that supplied in the roughage and the 12 per cent protein concentrate mixture.

TABLE 4

Summary of semen characteristics

	Semen characteristics					
	Vol.	Motility	No. of sperm per $mm.^3$	Total no. sperm per ejac.	Methylene blue reduction time	Discarded ejaculates
	(*ml.*)	(%)	$(\times 10^3)$	$(\times 10^6)$	(*min.*)	(%)
T.D.N. level fed						
100% mean	5.70	72.7	1,219	7,096	5.03	43.2
No. of ejaculates	216	179	197	197	161	215
120% mean	5.64	73.5	1,226	7,197	5.04	37.8
No. of ejaculates	281	244	249	249	216	281
140% mean	6.50	73.2	1,220	6,788	4.97	40.8
No. of ejaculates	246	211	219	219	186	246
Protein level fed						
12% mean	6.08	74.3	1,198	7,359	5.08	36.1
No. of ejaculates	246	214	220	220	203	246
16% mean	5.93	73.9	1,176	7,178	5.15	41.4
No. of ejaculates	248	209	225	225	178	248
20% mean	4.91*	71.2†	1,272†	6,544†	4.80	44.3
No. of ejaculates	248	211	220	220	182	248
Least significant difference for 2 selected means						
5% level	–‡	2.34	82	588	–	–
1% level	0.49	–	–	–	–	–

* Significant at 5% level of probability.
† Significant at 1% level of probability.
‡ – = Difference between treatment means not significant.

Fertility of the semen used. The fertility data were obtained only on those ejaculates meeting the relatively high standards of semen quality required by the New York Artificial Breeders' Cooperative, Inc. These data presented in summary form in table 5, show, by treatments, the total number of first services, the total number of 5 months' non-returns, and the calculated per cent of non-returns. Although there is a trend indicated by the results, especially with respect to the bulls fed the different protein

TABLE 5

Summary of fertility data

Treatments	Fertility criteria		
	Total first services	Total 5 months' non-returns	% non-returns
T.D.N. level fed			
100%	7,360	3,998	54.3
120%	9,419	5,168	54.9
140%	8,198	4,582	55.9
Protein level fed			
12%	10,190	5,863	57.5
16%	8,260	4,429	53.6
20%	6,527	3,456	52.9

levels, the wide variability in the fertility results of individual bulls makes these differences statistically insignificant.

Body weight changes. Mean initial body weights, mean body weight changes, and their standard errors are given in table 6.

Body weight changes at all levels of T.D.N. fed were significantly different from each other, while differences in body weight changes between protein levels were not. No significant T.D.N.-protein level interactions or T.D.N. carry-over effects were obtained.

A tentative feeding standard for mature dairy bulls. The relationship between the average body weight during two consecutive experimental feeding periods, in one of which there was a loss and in the other a gain in weight, and the estimated pounds of T.D.N. per day required to maintain this average weight are shown in figure 1.

TABLE 6

Mean initial body weights, mean body weight changes, and standard errors of the means for treatments

Treatment	Initial body weight	Body weight changes
	(*lb.*)	(*lb.*)
T.D.N. levels fed[*]		
100%	1,862 ± 85	−46.4 ± 9.2[†]
120%	1,770 ± 70	43.4 ± 9.3[†]
140%	1,767 ± 83	102.4 ± 11.8[†]
LSD[‡] 5% level		43.4
LSD 1% level		52.5
Protein levels fed		
12%	1,770 ± 163	135.7 ± 35.2
16%	1,736 ± 153	88.2 ± 26.6
20%	1,739 ± 117	83.3 ± 28.9

[*] Initial body weights for the T.D.N. levels represent the body weights at the beginning of the period in which the T.D.N. levels were fed. For protein levels the initial weight represents weights taken at the beginning of the experiment.

[†] Highly significant statistically.

[‡] LSD = Least significant difference.

The linear correlation coefficient, $r = 0.944$, and the linear regression coefficient, $b_{yx} = 0.006613$, as determined from the plotted values shown in figure 1, were both highly significant statistically. On the other hand, the deviations from linear regression were not significantly statistically. Therefore, the authors believe that the linear regression equation, $\hat{Y} = 2.33 + 0.006613\,X$ where $\hat{Y}$ is the pounds T.D.N. required at body weight X, furnishes a satisfactory basis for the establishment of a tentative feeding standard for mature dairy bulls.

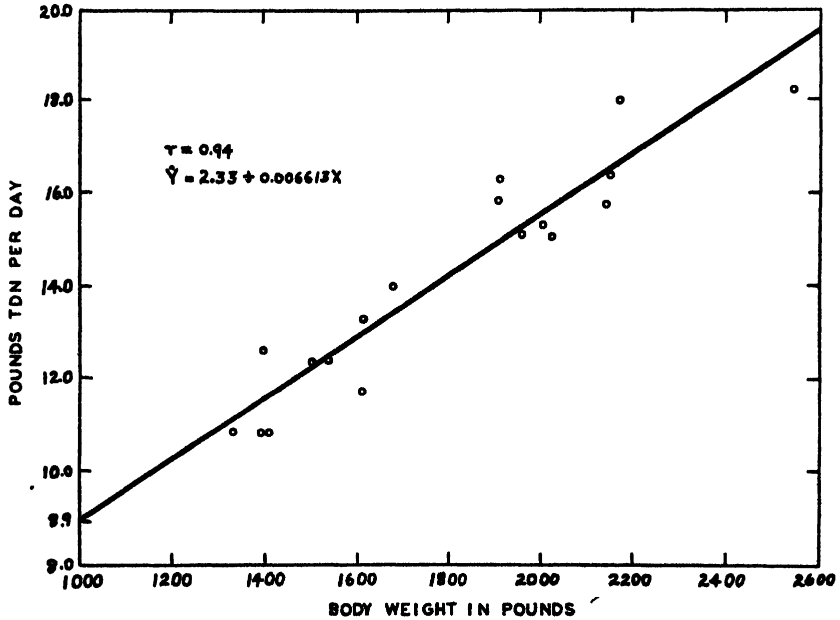

FIG. 1. Scatter diagram showing regression of pounds T.D.N. required per day on average body weight. (The points plotted were arrived at in the following manner: Average body weight = initial weight of the first period + final weight of the second period ÷ 2. The estimated pounds T.D.N. per day required to maintain the average body weight = pounds T.D.N. fed per day during the period in which there was a loss in weight increased by an amount equal to the fraction, pounds loss in weight ÷ pounds total change in weight, regardless of sign, × the difference in pounds T.D.N. per day fed during the two consecutive experimental feeding periods.)

Table 7 shows the calculated pounds of T.D.N. required per day for bulls of body weights from 1,200 to 2,600 lb. and the respective concentrates and hay equivalents when the concentrates furnish 40 per cent and the hay 60 per cent of the daily T.D.N. requirement.

In terms of a practical feeding schedule, the standard in table 7 reduces itself to the feeding of 0.46 lb. of concentrate mixture and 1.0 lb. of hay at the lowest body weight and 0.40 lb. of concentrate mixture and 0.9 lb. of hay at the greatest body weight per 100 lb. body weight per day. Furthermore, it appears that for mature dairy bulls a concentrate mixture containing approximately 12 per cent protein, when fed with mixed hay, will supply sufficient protein for maintenance of body weights as well as satisfactory semen production.

TABLE 7

*Tentative feeding standard for mature dairy bulls**

dy weight of bull (lb.)	Total lb. digestible nutrients required per bull per day	Pounds of 75% T.D.N. concentrate to supply 40% of total daily T.D.N requirement	Pounds of 50% T.D.N. mixed hay to supply 60% total daily T.D.N. requirement
1,200	10.27	5.5	12.3
1,300	10.93	5.8	13.1
1,400	11.59	6.2	13.9
1,500	12.25	6.5	14.7
1,600	12.91	6.9	15.5
1,700	13.57	7.2	16.3
1,800	14.23	7.6	17.1
1,900	14.89	7.9	17.9
2,000	15.56	8.3	18.7
2,100	16.22	8.6	19.5
2,200	16.88	9.0	20.3
2,300	17.54	9.4	21.0
2,400	18.20	9.7	21.8
2,500	18.86	10.1	22.6
2,600	19.52	10.4	23.4

* Based on the regression equation: $\hat{Y} = 2.33 + 0.006613X$, where $\hat{Y}$ = T.D.N. required at body weight X.

SUMMARY

Using 18 bulls, 9 Holstein-Friesians and 9 Guernseys, studies were conducted relating measurable semen characteristics, relative fertility, and body weight changes to T.D.N. intake levels of approximately 100, 120, and 140 per cent of recommended maintenance requirements for dry dairy cows of equivalent weights fed simultaneously with concentrate mixtures containing 12, 16, and 20 per cent total protein.

These relatively wide ranges of T.D.N. intake, accompanied by considerable shifts in body weight, can be fed mature bulls for at least 90 days without influencing semen production or fertility. Under the conditions of this experiment, 12 per cent of protein in the concentrate mixture fed with mixed hay, containing approximately 10 per cent of legumes, supplied enough protein for semen production and the maintenance of fertility.

Based on the results of this experiment, a tentative feeding standard for mature dairy bulls used routinely in artificial insemination is proposed. In terms of a practical feeding schedule, such a standard reduces itself to approximately 1 lb. of hay and 0.4 to 0.5 lb. concentrate mixture daily per 100 lb. of body weight.

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- Prussian Dairy Research Institute, Kiel, Germany
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- The Royal Technical College, Copenhagen, Denmark
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ABSTRACTS OF LITERATURE

BOOK REVIEW

1. **Modern Development of Chemotherapy.** E. HAVINGA, H. W. JULIUS, H. VELDSTRA, AND K. C. WINKLER. Edited by I. R. HOUWINK AND J. A. A. KETELAAR. 175 pages. $3.50. Elsevier Publishing Company, Inc., New York. 1946.

This publication represents one of a series of monographs on the progress of research in Holland during the war. These monographs, begun during the war, contain information most of which was kept secret until the end of the war.

The discussions include: (1) An introduction which emphasizes the important aspects of chemotherapy; (2) Bacteriological and physiochemical investigations on the mechanism of the action of the sulfonamides and of para-aminobenzoic acid; (3) Chemical investigations including synthesis and activity of sulfonamide derivatives and related compounds, action of sulfonamides on enzymes and analytical experiments on quantitative determination of sulfonamide derivatives and para-aminobenzoic acid; (4) Investigations on pharmacology, immunology, and clinico-therapeutical results including studies on streptococci, pneumococci, meningococci, gonococci, and other organisms; (5) Mycotherapy investigations on expansine, a product stemming from studies on antagonistic effect of antibiotics on plant pathogens.

The monographs are well written and presentations of data enhanced by numerous tables, figures, and structural formulas. P.R.E.

BACTERIOLOGY

2. **The Selective Action of Penicillin in the Isolation of *Brucella abortus* from Milk.** HELEN A. LACY, L. J. RODE, AND V. T. SCHUHARDT, Brucellosis Research Project of the Clayton Foundation, University of Texas, Austin, Texas. Jour. Bact., *52,* 3: 401. September, 1946. Abs. Proc. of Local Branches.

"Pooled milk samples from the four quadrants of the udder were collected in sterile test tubes by hand milking from 564 cows. The cream from these samples was plated in duplicate on the usual gentian violet tryptose agar (GVTA) and on this medium containing 1 Oxford unit of penicillin per cubic centimeter (PGVTA). *Brucella abortus* colonies were isolated a total of 87 times from the two media. In 58 instances *B. abortus* was isolated on the PGVTA and not on the duplicate GVTA. In only 1 instance was the reverse of this situation true. In 20 instances the PGVTA plates showed more than 50 *B. abortus* colonies, whereas not one of the GVTA plates showed more than 50 colonies. In 12 instances the PGVTA plate showed more than 50 colonies of *B. abortus,* whereas not a single colony was found on the duplicate GVTA plate. *B. abortus* was isolated from 86 (15.24 per cent) of the 564 samples on PGVTA and from 29 (5.14 per cent) of the samples on GVTA." D.P.G.

BUTTER

3. **Testing Sour Cream for Extraneous Matter.** Kenneth M. Renner, Texas Technological College, Lubbock, Texas. Southern Dairy Products Jour., *38,* 4: 64. October, 1945.

The following methods of filtering four-ounce samples of sour cream were used: (1) Soda Solution (Illinois Method), (2) Dry Soda Method, (3) Acid (HCl) Method recommended by Prof. M. G. Pederson of Texas Technological College, (4) Texas Tech. Method (Calgon–Diversey–USN–Citric Acid), (5) Special Solution designated by the Diversey Company as X–300, (6) Special Powder Solution, designated by the Diversey Company as D–229.

The first four tests have been recently described by the American Butter Institute. Method 5 consists in adding nine ml. of Solution X–300 to the cream sample, stirring for one minute, thoroughly stirring in eight ounces of water at 180–190° F. and filtering. For Method 6 make a 10% solution of D–229 in distilled water, heat to 160–180° F., stir frequently, allow to cool, and filter. Add two ounces of this stock solution to a sample, mix, allow to stand two or three minutes, add eight ounces of 180–190° water, mix well, and filter.

All methods filtered ordinary farm cream successfully except that Method 1 did not filter 35–50% cream five days old and Method 2 was not successful at five days for 50% cream and at seven days for 35–40% cream. When one ounce of a 50% solution of citric acid was added to each gallon of water used for diluting the cream, Methods 1 and 2 were successful on Grade A cream until it was at least seven days old. The use of untreated water with a hardness exceeding 15 grains caused difficulty in the alkaline methods.

Good results were obtained from Method 1 when the cream was allowed to stand three minutes after the addition of dry sodium bicarbonate, hot water added at 180° and the mixture stirred for three minutes before filtering.

Any differences between the methods in dissolving some of the sediment were not considered of material importance. Results were satisfactorily duplicated by each method provided the cream was thoroughly mixed before sampling. F.W.B.

CHEESE

4. **Paying for Swiss Cheese on a Solids and Fat Basis.** ARTHUR B. EREKSON, Plymouth, Wisconsin. Natl. Butter and Cheese Jour., *37,* 10: 48. October, 1946.

This method of payment is intended to give the producer of Swiss cheese an incentive to make cheese with optimum amounts of fat and moisture. It may also serve to protect the buyer who wishes to store cheese with high fat and low moisture and is willing to pay for it. Cheese with more fat or less moisture, or both, under this plan commands a proportionately higher price. Cheese with less fat or more moisture or both is worth less. It is calculated that cheese of optimum composition containing 61% dry matter, of which 45% is fat, has 0.2745 pounds of fat per pound of cheese. Cheese with the same dry matter, of which 46% is fat, contains 0.2806 pounds of fat and 0.3294 pounds of solids-not-fat per pound of cheese. This amount of solids-not-fat requires only 0.2695 pounds of fat to give the cheese the desired 45% fat in the dry matter so there is an excess of 0.0111 pounds of fat for which extra payment is needed. All of the value of the cheese price is assigned to the dry matter. The difference between the cheese-solids price and the price of butterfat in cream then is used to calculate the value of the amount of fat over or under the optimum. These calculations have been made and tabulated to show the value of a wide range of cheese compositions at the market price for Swiss cheese of 33 cents per pound. In order to sell the cheese beyond the primary market so that variations in purchase price will be fairly covered, it is recommended that the average compositions of cheese in each grade on the market be used to calculate the solids-plus-fat value of the cheese in those grades. W.V.P.

5. **A Discussion on Cheese Standards of Identity.** RAYMOND MIOLLIS, Natural Cheese Co., Maywood, Illinois. Natl. Butter and Cheese Jour., *37,* 11: 36. November, 1946.

The proposed "Standards of Identity for Cheese" is inadequate, especially for semi-hard and soft cheese of the imported type. The European nomenclature can be disregarded because the names are meaningless and obsolete for purposes of definition in the United States. The descriptions of manufacturing processes in the proposed standards are vague, erroneous and incomplete. Pasteurization of milk for all cheese should be compulsory and without the 60-day optional curing alternative. The amount of milk fat to be left in the cheese "should be left to the discretion of the cheesemaker, and after the cheese is made every cheese should be branded . . . with the butterfat content in multiples of five, followed by the sign +." Semi-hard and soft cheese should have no moisture limits. W.V.P.

ICE CREAM

6. Research on Milk Solids-Not-Fat. B. I. MASUROVSKY. Ice Cream Trade Jour., *42*, 6: 68. June, 1946.

Serum solids consist of milk protein, such as casein and albumen, which are used to build tissue and muscles. They also include the milk minerals to aid in the formation of bones and teeth. The average satisfactory serum solids in ice cream is about 11 per cent, depending upon the butterfat content of the mix. A high butterfat content requires less serum solids, but in no case should the combined milk solids exceed 22 per cent. Serum solids for ice cream are furnished by concentrated skim milk, plain or superheated; sweetened condensed skim milk; skim milk powder (spray process); and modified milk solids containing reduced quantities of sugar. Also, sweet buttermilk solids may be used. Skim milk used in the manufacture of skim milk solids should be tested for acidity, flavor, and sanitary quality.

W.H.M.

7. The Use of Processed Fruits, Nuts, and Other Flavors. E. C. WETMORE, Richardson Corp., Rochester, N. Y. Ice Cream Trade Jour., *42*, 6: 56. June, 1946.

Some of the advantages of using processed fruits, nuts, and other flavors in ice cream are uniform flavor and color in the finished ice cream, convenience in using, reduction of contamination of the ice cream because they are pasteurized after packing and sealing, and insurance of the maximum amount of flavor, because such fruits are usually selected and packed when they are at the right stage of ripeness. Nuts processed and packed in syrup retain their flavor and crispness in ice cream. Other flavors such as chocolate can also be obtained in sealed cans, and such flavors are usually uniform due to the ability of the processor to select the right blend of cocoa and chocolate liquor. W.H.M.

8. Powdered Ice Cream Mix. S. T. COULTER. Ice Cream Trade Jour., *42*, 5: 42. May, 1946.

Dry ice cream mix is processed by condensing the milk-cream mixture which has been adjusted to the desired ratio of fat to solids-not-fat. Stabilizer dissolved in hot water, color, and sugar are then added to the condensed milk, and the mixture spray-dried to about 1 per cent moisture.

The capacity of the drier can be increased by adding only a portion of the sugar to the liquid mix prior to drying, and then blending the remainder of the sugar with the dry mix. Vegetable stabilizers are preferred to those of animal origin. Glycerol monosterate, egg yolk solids, powdered buttermilk, and sodium caseinate improve the whipping properties of dry ice cream mix. The composition of dry ice cream mix varies, but the following is typical:

Fat	27–29 per cent
M.S.N.F.	26–27.5 per cent
Sugar	40–44 per cent
Stabilizer	0.6–1 per cent or more
Moisture	1.2 per cent

The milk products are usually preheated to 190° F. for 5 minutes or more or to a higher temperature for a shorter holding period, to retard oxidative deterioration. Prompt cooling of the powder to 100° F. or less before it is packed is desirable. Cooling decreases cooked flavor and the rate of development of stale flavor. Low moisture and oxygen levels and storage of the powder at a low temperature (below 70° F.) are the principal means of retarding the development of stale flavor. W.H.M.

9. **Ice Cream Through Countless Ages.** ANONYMOUS. Southern Dairy Products Jour., *38*, 4: 34. October, 1945.

Marco Polo during his journeys through the Orient in the fifteenth century became so fond of wines and sweetened water iced with snow from the mountains that he introduced the delicacy to his native Italy upon his return home. The Italians soon discovered that snow treated with saltpeter produced a mixture capable of freezing water ices.

About 1550 the Italians flavored the sweetened water with fruit juices and then substituted milk for water in the mixture. More and more cream was used. In the same century Catherine de Medici brought water ices into France and Italians set up water-ice shops in that country. Fancy molds were first introduced in 1774. The Germans introduced ice cream decorating as an art.

Ice cream was brought to England by Carlo Gatti from France, and King Charles I introduced it to his court. There are many conflicting stories as to who introduced ice cream to the United States. There are accounts of the serving of ice cream at the White House during the administrations of Andrew Jackson and James Madison and the product was advertised in New York and Baltimore 1786 to 1789 and in New Orleans in 1808.

The beginning of the wholesale manufacture of ice cream is credited to Jacob Fussell of Baltimore, Maryland, in 1850. The commercial manufacture spread to most of the other states during the remainder of the century.

Inventions of the Mojonnier milk tester in 1915, the ice cream overrun tester in 1917, the ice cream packaging machine in 1920, the Creamery Package 80-quart freezer in 1917, the ice cream cone in 1904, and the Eskimo Pie in 1921 were noteworthy contributions to the industry. Pennsylvania State College began instruction in the manufacture of ice cream in 1892 and Iowa State College in 1901. F.W.B.

10. The Use of Fruit Purees in Ice Cream. C. L. Bedford, Dept. of Horticulture, State College of Washington, Pullman, Washington. Ice Cream Trade Jour., *42,* 5 : 44. May, 1946.

Unsweetened fruit purees may be made by passing such fruits as raspberries, loganberries, boysenberries, youngberries, strawberries, Santa Rosa plums, and similar fruits through a tomato-juice extractor or a finisher such as is used to remove peel from cooked squash or pumpkin. The fruits should be pureed at 40–50° F. and then frozen. A deaerator may be used to remove air. This prevents changes in color and flavor during freezing, storage, and thawing. Peaches, apricots, and pears should be scalded or steamed for 4 to 5 minutes to prevent darkening when exposed to air.

Fruit purees may also be prepared by adding sugar or syrup to the puree. Two volumes of berries, sour cherries, plums, and strawberries may be frozen with 1 volume of 67% syrup; apricots and nectarines, 1 volume pulp to 1 volume of 50% syrup; and blackberries, Bing cherries, pears, 3 volumes pulp to 1 volume of 67% syrup.

Other types of fruit purees contain gelatin or pectin, and they may be used in making fruit ribbon, ripple, or marble ice cream, or in making a product developed by the Western Regional Research Laboratory called "Velva Fruit".

The formula for "Velva Fruit" is varied slightly for different fruits, but two typical formulas for 100 gallons of mix are given.

For unsugared fruit puree with high acid and low pectin content—such as raspberries, boysenberries, loganberries, youngberries, Santa Rosa plums, strawberries, and similar fruits:

Puree	640 lbs.	610 lbs.
Sucrose	265 lbs.	170 lbs. (high-conversion corn sirup—125 lbs.)
Gelatin (275 Bloom)	5 lbs. 13 ozs.	5 lbs. 13 ozs.
Water	60 lbs.	60 lbs.

For fruits with low acid and high pectin content, such as unsugared apricots, pears, peaches, and similar fruits:

Puree	680 lbs.
Sucrose	225 lbs.
Gelatin (275 Bloom)	5 lbs. 13 ozs.
Water	60 lbs.
Citric acid	1 lb. 14 ozs.

In the high-acid fruits the soluble solids content should be about 37 to 38 per cent with about 1 part sugar to 2.4 parts of fruit puree. Corn sirup can be substituted for one-third of the sugar. It must be added in a 3 to 2 ratio to maintain the same sweetness.

In the low-acid fruits a soluble solids of 34 to 35 per cent and a sugar-fruit ratio of 3 to 1 is satisfactory.

In the preparation of the mix, the puree, sugar, and citric acid (if used) are mixed together until well dissolved. This should be kept relatively cool. The gelatin is mixed with ten times its weight of water and is heated to 170 to 180° F. to dissolve and "sterilize" it. During the addition of the gelatin sol the mix is stirred in order to prevent the formation of stringy gelatin in the mix. If a batch freezer is used, the gelatin sol can be stirred into the mix in the freezer before the refrigerant is turned on. W.H.M.

11. **Blower-Type Evaporators in Ice Cream Plants.** C. H. MINSTER. Ice Cream Trade Jour., *42,* 8: 34. August, 1946.

The blower type evaporator for ice cream plants has several advantages over the conventional unwieldy multiple-coil evaporator. It takes up less space, hardens ice cream faster, permits use of higher back pressures, lowers operation cost, is easily moved from one location to another, is easily defrosted, and requires little time for installation.

Two common troubles encountered in the operation of blower evaporators are frost on the coil and oil congealing in the float, surge drum, or coils. Most low-temperature units are equipped with a device for defrosting with calcium chloride brine. Sometimes these defrosters give trouble because of clogged brine spray, failure to close suction and liquid valves, weak brine, tendency to stop brine circulation before coils are completely defrosted, and the tendency to defrost only when frost has built up to a point where the circulation is reduced to a minimum. Heating the brine tended to offset these difficulties.

Some of the problems encountered with oil are: (1) Oil congeals in the float-valve orifice, preventing ammonia from entering the coils. Result: The evaporator would pump out and, with no refrigerant in it to absorb heat, all refrigeration would stop. (2) Oil, passing through the float-valve orifice with the float-open, would congeal as soon as it hit the lower temperature of the float housing. Here it would build up and support the float in an open position. Result: Ammonia entered the coils in a steady stream and in quantities in excess of that required to maintain proper ammonia level. This ammonia would flood back on the compressor. (3) Oil would gradually accumulate and congeal in the bottom coils of the evaporator, resulting in reduced or completely shut off entrance of ammonia to coil, which further resulted in either refrigerant starvation or complete pump-out of the coils, thus slowing or stopping refrigeration. (4) The bottom coils became filled with fluid oil, resulting in reduced efficiency and heat transfer, and impediment of ammonia travel.

Remedies: (1) Maintain coils frost-free. (2) Constantly circulate air (from the room to be cooled) over the coils and back to the room. (3) Maintain the highest possible back pressure within the coils. (4) Keep the entire unit oil-free.

Low-temperature blower type evaporators work better if installed in a separate room from the room to be refrigerated. Some of the advantages of such an installation are: (1) Maintenance work on the unit can be more readily performed. (2) Defrosting can be accomplished with plain water. (3) The unit can be warmed up while defrosting with a minimum rise in hardening room temperature. (4) Oil can readily be drained from the unit.

W.H.M.

12. **Ice Cream with Less Sugar.** E. L. FOUTS, Dairy Technologist, Florida Experiment Station, Gainesville, Florida. Southern Dairy Products Jour., *38*, 5: 96. November, 1945.

In 1943 the Florida Dairy Products Laboratory published Bulletin No. 393, entitled "The Preparation and Use of Invert Sirup in the Manufacture of Ice Cream." In this bulletin, it was shown that it was possible to increase the sweetening power of cane or beet sugar by inversion.

It is possible to lower the sugar content of ice cream to 12% or even less by increasing the serum solids proportionately. For example, if ice cream is being made with 10.5% fat, 11% serum solids, 14% sugar, and 0.25% stabilizer, the sugar can be reduced to 12% with a corresponding increase in serum solids from 11% to 13%, producing an ice cream which is quite satisfactory in sweetness and in other respects. Trials have shown that the serum solids content can be increased to 13.5% if necessary. The inversion of not more than 25% of the sugar also will tend to give increased sweetness. The use of invert sirup in excess of 25% of the total sugar lowers the freezing point of an ice cream mix so that it may be difficult to harden.

It has been proven that the degree of sweetness in ice cream is influenced by the amount of water in the ice cream mix. This may be a partial explanation of why the sugar content can be reduced when the serum solids contribute additional milk sugar. Actually, then, when the sugar content is reduced from 14 to 12% and the serum solids increased from 11 to 13%, the sucrose content is reduced 2% but in turn the lactose content is increased 1%. If part of the sugar is inverted, the serum solids should be increased to maintain the same total solids in the mix, since the invert sugar sirup contains only about 70% sugar solids.

The additional serum solids seem to cover up or absorb vanilla flavor. It is suggested that a little extra vanilla be added when increasing the percentage of serum solids. To reduce the sugar content of ice cream enough to be worthwhile without making other changes will result in a thin coarse-textured ice cream definitely lacking in sweetness and flavor. F.W.B.

13. Chocolate Ice Cream. C. D. DAHLE, Pennsylvania State College. Southern Dairy Products Jour., *38*, 5: 28–30. November, 1945.

A sirup of two pounds of cocoa, two pounds of sugar and enough water to make a gallon of sirup is sufficient to flavor 10 gallons of finished ice cream. The reduction in the fat content of the mix from the addition of the sirup must be taken into account. The fresher the sirup the better is the flavor of the ice cream.

The flavor, body and texture of chocolate ice cream are improved by making a chocolate mix. The main drawback is that frequently the viscosity of the mix is so increased that cooling is difficult. The causes of high viscosity of the mix are: (1) stabilizer, (2) pressure, (3) acidity, (4) amount and kind of chocolate, (5) composition of mix, and (6) pasteurizing temperature.

Certain stabilizers cause more trouble than others. Reduce the amount used 20 to 30 per cent or more.

Single-valve homogenization will cause greater viscosity than "two-stage". The pressure on the single valve may be reduced from 2500 pounds to 1500 or less and in two-stage homogenization to 1500 and 500 pounds on the respective valves. The temperature of pasteurizing and homogenizing should be at least 160° F.

The acidity of the unflavored mix should be standardized and vary from about 0.15% for 8% M.S.N.F. to 0.20% for 12% M.S.N.F.

The more chocolate used the thicker and darker will be the product. The normal amount is 3–3½ per cent. The flavor is carried by the cocoa solids-not-fat and not the fat. The amount of each chocolate product needed to flavor 10 gallons of ice cream is about as follows: chocolate liquor (50% cocoa fat), 3.0 lbs.; blend (35% fat), 2.3 lbs.; cocoa (25% fat), 2.0 lbs.

Chocolate ice cream contains more sugar than vanilla ice cream to reduce the bitterness from the flavor. The more cocoa used, the more sugar is needed. A lower cocoa content may be compensated by a reduction in sugar to make the cocoa "taste stronger".

Chocolate is a relatively inexpensive flavor. In 1938, it was used in flavoring 16.36% of the ice cream and was surpassed only by vanilla, used in 51.26%.

Variegated chocolate ice cream may be made by adding a thick sweetened sirup to the ice cream as it is discharged from the freezer. The sirup may be purchased or made in the plant to contain 45–50% sugar, 1% stabilizer and 15% cocoa.

Sweetened slab chocolate may be melted at 105° F. and added to the ice cream as it comes from the freezer to give the ice cream a specky appearance. The colder the chocolate and the ice cream, the larger will be the pieces.

(The original article appeared in Serum Solids Digest and was reprinted by permission of Prestige Products Company, New York.) F.W.B.

14. Frozen Food Packaging—A Preliminary Study of Cavity Ice. C. I. SAYLES, W. A. GORTNER, AND FRANCES E. VOLZ, School of Nutrition, Cornell University, Ithaca, New York. Food Freezing, *1,* 11: 430. September, 1946.

The term "cavity ice" is suggested to designate the formation of ice in the air spaces inside a package of frozen food stored in a cabinet. The condition is found even though water-vapor transmission is at a minimum because of protective covering. The condition arises from dehydration of the food within its protective covering, the resulting withdrawn moisture being deposited as ice crystals within the cavities of a loosely packed product like snap beans or on the surface of a solid product like meat. The authors attribute the phenomenon in part to radiant heat transfer of moisture from the food to the air within the package. Interposition of foil prevented the formation of cavity ice because of its radiant heat transfer barrier between the package and side wall plates. Maintenance of temperature uniformity is highly important in order that the temperature gradient within the package will be small, thus keeping down moisture abstraction from the food. Insulation of package was found to be effective in keeping down temperature fluctuation but at present would be prohibitive in cost. In general, cabinet design which would favor a higher humidity level than that commonly found would prevent moisture transferal. Also, keeping down the temperature change of refrigerated surface in cycling will aid materially. Seventeen illustrations and three diagrams. L.M.D.

15. Frozen Food Stores. VINCENT M. RABUFFO. Ice Cream Trade Jour., *42,* 7: 26. July, 1946.

The Ice Cream Trade Journal has made an ice cream sales survey in specialized frozen food stores in 17 eastern and midwest communities. It was found that the volume of sales ranged from 5 gallons to 300 gallons a week. All stores sold ice cream made by commerical manufacturers and only one store expressed a desire to make its own ice cream. Sixty per cent handled a deluxe high-fat ice cream, while 40% handled the regular commercial grade. Packaged ice cream was sold by 95% of the stores. The other 5% handled both bulk and packaged ice cream. Most of the stores were not interested in dipping ice cream. The price on deluxe ice cream ranged from 38 cents to 50 cents per pint, and standard grades averaged 30 cents per pint. Most of the deluxe grade sold for 45 cents per pint.

The price paid to manufacturers for the regular grade ranged from $1.25 to $1.60 per gallon, with most of them paying $1.50. These prices prevailed during July before any general advance went into effect. The wholesale price for the deluxe grade ranged from $2.00 to $2.85 per gallon.

Seventy-seven per cent of the stores handling the deluxe grade had a mark-up of 50% or more. It has been established that a good quality ice cream at a good price can be sold by these stores. These mark-ups have been higher than those enjoyed by other retail outlets on ice cream. W.H.M.

PHYSIOLOGY

16. **The Relation of Temperature and the Thyroid to Mammalian Reproductive Physiology.** RALPH BOGART AND DENNIS T. MAYER, Missouri University, College of Agriculture, Columbia. Amer. Jour. Physiol., *147,* 2: 320–328. October, 1946.

High temperatures cause a marked lowering in the activity of the reproductive organs of rams.

Thyroxine or thyro-active proteins given to rams during periods of high temperature stimulate the reproductive organs and restore most of the reproductive activities to a level near that of the breeding season.

Changes in semen characteristics similar to those resulting from high environmental temperatures are induced during the breeding season by administration of thiouracil. Thyro-active materials counteract the detrimental effects of thiouracil upon reproduction.

Since the temperatures which will reduce fertility in the ram are below the temperature at which the testes normally function, and since thyro-active materials restore reproductive activity during periods of high temperature, it is concluded that temperature is not influencing reproductive physiology by its direct effect on the testes.

The stimulating effect of thyroxine during periods of high temperatures and the harmful effect of thiouracil administered during the normal breeding season suggest that the level of thyroid function influences the relative activity of the reproductive organs. D.E.

MISCELLANEOUS

17. **Styrofoam—A New Thermal Insulation.** O. R. MCINTIRE AND D. W. MCCUAIG, The Dow Chemical Company, Midland, Michigan. Refrigerating Engineering, *52,* 3: 217. September, 1946.

A polystyrene foam, "Styrofoam", now being produced in limited quantities, is an extremely light plastic structure composed of many small plastic bubbles, each bubble being completely enclosed to make the entire plastic structure impermeable. Styrofoam 103.7 has a K factor of 0.27 to 0.29 and cell sizes of 1/32 to 1/16 inch. Experimentally a product with aluminum powder incorporated in it has a K factor of 0.20 due to decreased infrared transmission. Styrofoam has in addition the following desirable factors: low specific gravity, low water absorption, high resistance to vapor diffusion, structural strength yet bendable, easily installed in sheets. Because it is made from polystyrene, a thermoplastic, Styrofoam begins to distort upon continuous exposure to temperatures above 180° F. but at low temperatures it becomes tougher and stronger. Installation procedure in commercial applications such as cold storage plants can be followed as for corkboard with the single precaution that asphalt temperature must be kept low enough (not over 175° F.) to prevent the Styrofoam from softening. Due to its very light weight it lends itself to the insulation of mobile equipment where the minimizing of weight is of prime importance. It should prove to be of exceptional value in the field of frozen foods transportation where the maximum of insulation effect is needed to hold low temperatures together with a maximum of goods weight and maximum of cargo space. L.M.D.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

18. The Origin and Control of Thermoduric Organisms. Some Fundamental Phases. DAVID LEVOWITZ, New Jersey Dairy Lab., New Brunswick, N. J. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 219. 1945.

Thermoduric bacteria are present in milk freshly drawn from normal, healthy cattle. Research has not established whether poor health of cows raises their numbers. The cow does not affect the thermoduric content of milk enough to affect municipal milk standards. Bedding, manure, and feed dusts are sources of thermoduric bacteria. The sediment test would begin to mean something if straining of milk on the farm were eliminated. Contamination of milking machines with thermoduric bacteria may become important when machines are not handled properly.

Although thermoduric bacteria are not pathogens, they are associated with unclean equipment surfaces and should be held at minimum numbers. It is difficult to sterilize film-coated surfaces. High-temperature, short-time pasteurization cannot yield thermoduric counts as low as the holder method.
A.C.D.

19. Significance of Thermoduric and Thermophilic Bacteria in Milk and Their Control. F. W. FABIAN, Division of Public Health, Michigan State College, East Lansing, Mich. Jour. Milk Technol., *9*, 5: 273–278. Sept.–Oct., 1946.

Thermophilic bacteria are more resistant than thermoduric organisms. They are distributed widely in feeds, grain, soil, cow hairs, manure, and improperly cleaned utensils. These organisms may contaminate the dairy plant from producers' milk and be propagated in the pasteurizing equipment, if it is not properly cleaned. These groups of organisms are not pathogenic. Their presence in appreciable numbers in milk indicates unsanitary practices both on the farm and in the milk plant. H.H.W.

20. Heat Resistant Bacteria from an Unclean Milking Machine Invade the Udder of the Cow. C. S. BRYAN, H. S. BRYAN, AND KARL MASON, Michigan Agricultural Experiment Station, East Lansing, Mich. Milk Plant Monthly, *35*, 8: 30–32. 1946.

The properly pasteurized milk from a small dairy increased during a 4-month period from its normal level of 15,000 to as high as 2,500,000 on some days. Despite careful plant cleaning and sanitizing, the high count continued. The source of the high count of heat-resistant bacteria was found to be the milk of one of the seven producers. An unclean milking machine contributed heat-resistant bacteria to the milk, both directly during the milking process and indirectly by inoculating the cows' udders with these bacteria. The cows were free of the heat-resistant bacteria in periods varying from 1 to 4 months after the milk equipment was properly cleaned and sanitized. During this same period, the standard plate count of the properly pasteurized milk of this dairy decreased to its previous level of 10,000 to 25,000. G.M.T.

21. Thermal Death Range of Bacteria in Milk. A New Electric Sampling Device. F. W. GILCREAS AND J. E. O'BRIEN, New York State Dept. of Health, Albany, N. Y. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 237. 1945.

Study of the destruction of pathogenic bacteria by temperatures higher than now employed in the dairy industry is needed. Recently the Trumbull Electric Co. of Plainville, Conn., constructed a mechanism, based upon ideas of C. W. Weber, which makes possible laboratory pasteurization with accurate one-second holding intervals. The equipment is electrically activated and adds culture and withdraws samples by syringes. Tests were made on one ordinary *Escherichia coli* culture and two heat-resistant strains from the U. S. Public Health Service. It was concluded that the thermal death point for *E. coli* is not constant, and "therefore only a thermal death range based on results of repeated tests with many cultures can be determined."

A.C.D.

22. The Survival of Staphylococci Food Poisoning Strain in the Gut and Excreta of the House Fly. SARAH MOOREHEAD AND HARRY WEISER, Dept. of Bacteriology, Ohio State University, Columbus, Ohio. Jour. Milk Technol., *9,* 5: 253–259. Sept.–Oct., 1946.

A food-poisoning strain of staphylococcus, *Staphylococcus aureus* 611, suspended in a dilute sucrose solution, was fed to 900 "cultured" houseflies, *Musca domestica.* The test organism was recovered from the digestive tract in some of the flies periodically through 8 days.

Staphylococci not of a food-poisoning strain were isolated from the digestive tract of 10 of the 50 wild-caught flies examined. This indicates that staphylococci may be commonly carried by flies.

Musca domestica may serve as a reservoir host for *Staphylococcus aureus* 611, and under suitable conditions the fly may initiate or augment a food-poisoning outbreak by spreading staphylococci from infected handlers or dirty equipment to food and from contaminated supplies to good foodstuffs which are favorable for enterotoxin production. The organism may survive in the digestive tract of the housefly several days after contamination and be deposited on food even after the carrier source has been removed or after the fly has sought a new feeding location. H.H.W.

23. The Influence of Surface Active Cationic Germicides on the Bacterial Population of Milk. Adrien S. DuBois and Diana D. Dibblee, Onyx Oil and Chemical Co., Jersey City, N. J. Jour. Milk Technol., *9*, 5: 260–268. Sept.–Oct., 1946.

Alkyldimethylbenzylammonium chloride did not influence the bacterial counts of raw or pasteurized milk at concentrations ranging from 1: 500 to 1: 25,000. The higher concentrations had an inhibitory effect on the growth of Gram-positive acid-producing organisms but did not affect the Gram-negative bacteria. When this compound was used in lower concentrations, no such effect was noted. A chemical method for the estimation of surface-active cationic germicides in milk and a qualitative test for their detection in solutions are described. H.H.W.

BUTTER

24. Experiments on the Packing and Storage of Butter. Part V. The Effect of the Temperature-Level of Storage on the Keeping Quality. C. R. Barnicoat, Dairy Res. Inst., Palmerston North, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 4: 343–348. Dec., 1945.

At storage temperatures between 14° and 60° F., the average rate of deterioration of well-made sweet cream butter, as measured by decrease in the score for flavor, was related directly to both time and temperature. The average rate of loss in score increased by about 0.15 point per week for each 10° F. rise in temperature above 14° F.

The slight advantage obtained by storage at – 5° F. instead of 14° F. was not warranted by the extra cost. W.C.F.

25. Some Experiments on the Use of Parchfoil and Pliofilm for the Wrapping of Butter in *Pinus radiata* Boxes. F. H. McDowall, Dairy Res. Inst., Palmerston North, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 4: 303–308. Dec., 1945.

A strong wood flavor developed within 10 days in butter wrapped in parchment and packed in boxes made of *Pinus radiata.* Wrapping in parchfoil (aluminum foil between parchment sheets) prevented the development of primrose color on the surface and wood taint in the butter up to 2 years, except where the wrappers joined. Pliofilm wrapping prevented the color defect but permitted the absorption of the flavor within 6 months. The treatment of the box with pliowax did not prevent the taint. Tensilized pliofilm was not satisfactory. Little trouble with mold growth was encountered, but under commercial conditions molds might appear under the pliofilm. W.C.F.

26. **Measurement of the Gas Content of Concentrated Butter and Other Fat Products.** G. L. HILLS AND J. CONOCHIE (Div. Indust. Chem.). Austral. Council Sci. & Indus. Res. Jour., *18,* 4: 366–372. Nov., 1945.

A modification of the method of Rahn and Mohr is described.
W.C.F.

27. **The Manufacture of Dry Butterfat and of "Butter Concentrated Hardened."** W. J. WILEY AND G. W. COOMBS (Dairy Research Section). Austral. Council Sci. & Indus. Res. Jour., *19,* 1: 140–146. Feb., 1946.

Methods for large-scale production are described. W.C.F.

28. **Experiments on the Manufacture and Storage of Ghee.** C. R. BARNICOAT, Dairy Res. Inst., Palmerston North, New Zealand. New Zeal. Jour. Sci. and Technol., *27A,* 4: 309–319. Dec., 1945.

Attempts to produce ghee from cow butterfat yielded a product resembling Indian ghee but not entirely characteristic. Dry New Zealand butterfat was found to be acceptable to the Indian trade. These products when canned kept fairly well over 9 years of storage at 40° F. W.C.F.

CHEESE

29. **The Effect of Hydraulic Pressing on Cheese Texture.** H. R. WHITEHEAD, Dairy Res. Inst., Palmerston North, and L. J. JONES, Dairy Div., Dept. of Agr., Wellington, New Zealand. New Zeal. Jour. Sci. and Technol., *27A,* 5: 406–410. Feb., 1946.

When pressing cheddar cheese by hand-screw presses was compared with pressing by hydraulic presses, use of the latter almost eliminated "mechanical" openness in normal cheese of good quality but did not influence "slit" openness which may develop later. W.C.F.

CHEMISTRY

30. **Surface Chemistry in Chemical Cleaners.** GEORGE J. LEHN, Turco Products, Inc. Milk Plant Monthly, *35,* 7: 50–53. 1946.

Much time is spent in eliminating soil, such as film, scale, rust, and casein accumulations, from surfaces of dairy equipment. New knowledge of cleaners points more toward surface chemistry, which involves destroying the bond holding the soil to the surface. Wetting action brings cleaning solutions into close contact with oily, fatty, adhesive films, in order that soil may be dislodged more easily. Better wetting is made possible by the control of surface and interfacial tensions. Cleaning a surface involves wetting, emulsifying, saponification, and solvent action. Carefully formulated cleaners are buffered so that their cleaning energy will be retained. Also, properly formulated cleaners condition and control the precipitation of minerals in hard water, thus assuring free and complete rinsing. Applications of surface chemistry to cleaners should save enormous amounts of production time and costs in dairy sanitation. G.M.T.

31. The New Non-Chlorine Disinfectants. D. H. JACOBSON. Ice Cream Field, *48,* 4: 70. Oct., 1946.

Quaternary compounds are compared with chlorine compounds as germicides. The following advantages of quaternary compounds in dairy plants are listed: (a) non-corrosive on common metals, (b) non-toxic, (c) stable under heat and over long periods of time in common dilutions, (d) non-irritating to the skin, (e) colorless and odorless in common dilutions, (f) rapid wetting and penetrating action, (g) relatively non-selective towards various types of bacteria, (h) surfaces may be rendered bacteriostatic for some time after treatment, (i) not affected by hard water salts.

The organization of the "National Sanitation Foundation" in 1945 in the School of Public Health at the University of Michigan is mentioned. This organization is supported by the industry, and expectations are that the information required for developing the use of the products in dairy plants will be supplied. Brief mention is made of some experimental results already published by other workers. W.C.C.

32. The Use of Quaternary Ammonium Compounds in the Dairy Industry. C. A. LAWRENCE, Winthrop Chemical Co., Rensselaer, N. Y. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 177. 1945.

The quaternary ammonium compounds are surface-active agents consisting of two parts, a hydrophilic group and a lipophilic group. Since the lipophilic group is charged positively, these compounds also are known as cationic sterilizers. The wetting action, degree of detergency, and lowering of surface tension do not in themselves determine sterilizing action. The cationic sterilizers are effective for both Gram-positive and Gram-negative bacteria. These are most effective in alkaline solutions.

The cationic sterilizers are non-corrosive, non-toxic, odorless and non-irritating to the skin. They have high phenol coefficients. High concentrations of organic matter reduce their efficiencies. Soap and other anionic detergents also reduce their efficiency. These cationic compounds (alkyldimethylbenzylammonium chloride known as Zephirol, Zephiran, and Roccal was given as an example) have been found to be effective in sterilizing dairy equipment. A.C.D.

33. **Higher Fatty Acid Derivatives of Proteins.** W. G. Gordon, A. E. Brown, and R. W. Jackson. Eastern Regional Res. Lab., U. S. Dept. of Agr., Philadelphia 18, Pa. Indus. and Engin. Chem., Indus. Ed., *38*, 12: 1239–1242. Dec., 1946.

Casein and other proteins were modified by preparing a series of novel fatty-acid derivatives by the reaction of acid chlorides with the proteins dissolved in aqueous alkali. The procedure developed gave derivatives of casein which were acylated to the extent of approximately 20% by substituent groups ranging from caprylyl to steoroyl. The physical and chemical properties of palmitoyl casein are discussed. The acylated products show reduced affinity for water and altered solubilities. B.H.W.

34. **Plastic Properties of Higher Fatty Acid Derivatives of Proteins.** W. G. Gordon, A. E. Brown, C. M. McGrory, and E. C. Gall, Eastern Regional Res. Lab., U. S. Dept. of Agr., Philadelphia 18, Pa. Indus. and Engin. Chem., Indus. Ed., *38*, 12: 1243–1245. Dec., 1946.

Data on the water absorption and tensile and flexural strengths of molded test specimens of the higher fatty-acid derivatives of casein and other proteins are compared with similar data for casein hardened with formaldehyde. The acylated casein molding powders flow well in small positive type molds, and the molded pieces do not require further treatment with formaldehyde to yield finished articles. The molded specimens were light to dark yellow and translucent, with many almost transparent. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

35. **Keeping Qualities of Whole Milk Powder and Oatgum Mixes.** H. A. Bendixen, Division of Dairy Husbandry, Washington State College, Pullman, Wash. Ice Cream Field, *48*, 4: 68. Oct., 1946.

Comparisons of oatgum and sodium alginate as stabilizers for ice cream are reported. Whipping ability, flavor, and texture were similar when 0.5% of the former and 0.22% of the latter were used. A gravimetric method of determining solubility of milk powder, developed at the Washington Experimental Station, is mentioned. W.C.C.

36. **The Keeping Quality of Australian Milk Powders.** C. C. Thiel and E. G. Pont (Dairy Research Section). Austral. Council Sci. & Indus. Res., *18*, 4: 373–390. Nov., 1945.

Gas packing did not improve materially the storage life of skim milk powders but greatly increased that of whole milk powders. Because of leakage of cans, the results of gas packing on a commercial scale often were nullified. The influence of different storage temperatures tried, 15, 30, and 37° C., was not marked. No correlation was observed between bacterial counts and the initial or final quality of the powder. Working fresh butterfat into stored skim milk powder gave a product no better than the stored, gas-packed, whole milk powder. W.C.F.

37. **Studies on Compressed Whole Milk Powder.** C. C. THIEL (Dairy Research Section). Austral. Council Sci. & Indus. Res., *18,* 4: 391–406. Nov., 1945.

The compression of spray-dried whole milk powder to a density of 1.15 to 1.2 reduced the interstitial oxygen as effectively as did the usual gas packing in cans, but neither cellophane nor waxed paper wrapping prevented the uptake of atmospheric oxygen or moisture. Blocks made of the milk powder containing 20% of cane sugar remained more friable and kept better than blocks made up of milk powder alone. Added vanillin improved the keeping quality of the milk powder in blocks. W.C.F.

38. **Cultured Dairy Products, Production and Quality Control, Part I . . . . Buttermilk.** S. M. MANN, General Biochemicals, Inc., Chagrin Falls, Ohio. Milk Plant Monthly, *35,* 8: 26–29. 1946.

A brief review of literature on the development of dairy cultures and on some of the salient facts pertaining to the development of a high-quality cultured buttermilk is given. The cardinal principles to observe in the handling of milk cultures are: 1. Use best-quality milk; 2. Work cleanly and carefully in clean, draft-free surroundings; 3. Use clean utensils, preferably those which have been sterilized or rinsed in chlorine (20–30 p.p.m.) solutions; 4. Strictly observe sterilization, pasteurization, incubation, and cooling temperatures.

The production of high-quality cultured buttermilk involves: (a) pasteurizing milk at 185 to 190° F. for 30 minutes; (b) cooling and setting to 70° F.; (c) adding 4 gal. of 20% cream to 100 gal. skim milk; (d) adding 1 to 2% starter; (e) setting for 16 hours at 70°; (f) salting at the rate of 0.5 oz. per 10 gal., and adding highly colored butter granules if desired, after which the cultured milk is churned at high speed 5 to 10 minutes; (g) drawing off the churned cultured buttermilk into a coil vat and cooling to below 40° F. G.M.T.

DISEASES

39. **Mastitis Prevention.** I. E. PARKIN, Pennsylvania State College, State College, Pa. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 187. 1945.

Mastitis can be eliminated almost entirely from our dairy herds, even though research at Pennsylvania State College has shown that mastitis-producing bacteria are present in the udder of practically every cow. The problem is primarily one of best herd-management practices.

Factors of major importance are good barns, well-bedded stalls of ample size, feeds of high vitamin A potency, prepartum milking of cows whose udders are inflamed, and the new managed-milking program. One of the greatest preventatives of mastitis is the use of managed-milking practices, which are described briefly. Several instances of marked improvement of mastitis by the managed-milking program were cited. A.C.D.

HERD MANAGEMENT

40. The Effect of the Level of Stimulus Applied by the Pulsator on the Rate of Machine Milking. W. G. WHITTLESTON, Animal Res. Sta., Dept. of Agr., Ruakura, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 5: 445–450. Feb., 1946.

Under otherwise normal conditions of machine milking, the application of a greatly reduced pulsator stimulus had no effect on the milking rate. Once the milk flow starts with the pulsator operating, it will continue if the pulsator is stopped. Normal milk letdown is not obtained, however, if an attempt is made to milk without the pulsator when the teat-cups are applied. W.C.F.

ICE CREAM

41. How Golden State Cuts-Wraps Slices. GEO. D. AMERDING, Mojonnier Bros. Co. Ice Cream Field, *48*, 4: 76. Oct., 1946.

The cut-wrap slice machine is claimed to give the best and most popular individual serving of ice cream. The machine will produce 5,000 slices per hour, which is equal to 200 gallons of ice cream if cut seven slices to the quart. With waxed paper costing $0.13 per lb., waxed liners for cartons will cost $0.241 per M. Using the cut-wrap slice machine, labor costs for slicing one gallon of ice cream would be about 2.5 cents. The cost of the individual slice of ice cream is lower than any other individual package so far introduced. W.C.C.

42. Manufacture of Dry Ice Cream Mix. S. T. COULTER, Dairy Division, University of Minnesota, St. Paul, Minn. Milk Plant Monthly, *35*, 7: 84-85. 1946. Also Ice Cream Field, *48*, 4: 56. Oct., 1946.

Dry ice cream mix developed during the war has some advantages over liquid mix for ice cream manufacturers who buy mix. Transportation costs are lower, refrigeration unnecessary, and the mix is less perishable. Dry ice cream mix often is subject to oxidation during storage, the rate of oxidation depending upon such factors as freshness of dairy products used, degree of preheating of the milk, the presence of metallic catalysts, and temperature of storage. The moisture level of the dry mix seems to be a persistent factor influencing staling. Keeping quality of the dry mix may be improved by reducing the moisture level as low as 1%, and avoiding storage at unduly high temperatures. Composition of dry ice cream mixes and steps in processing the dry ice cream mix are given. G.M.T.

43. **Analysis of Stabilizers.** C. D. Dahle, Pennsylvania State College, State College, Pa. Ice Cream Field, *48*, 4: 62. Oct., 1946.

Gelatin, sodium alginate, locust bean (carob gum), Irish moss, sodium carboxymethyl cellulose, oatgum, pectin, Karaya, and Psyllium seed husks (ground) as stabilizers in ice cream are discussed. Because of the shortage of certain stabilizers during the war, several "mixed" stabilizers were placed on the market. These often contain various sugars which aid in dispersing the stabilizing agent and also may contain one or more of the following: sodium bicarbonate, sodium citrate, whey powder, milk solids dextrines, and, possibly, emulsifying agents. Emulsifying agents such as glycerol monostearate, sodium mono-palmitate, and sorbitan monostearate are mentioned briefly as aids to the whipping ability of ice cream mixes. W.C.C.

44. **Flavors and Fruits from Brazil.** H. A. Cardinell, Horticultural Section, and P. S. Lucas, Dairy Section, Michigan State College. Ice Cream Field, *48*, 4: 58. Oct., 1946.

Results of experiments in which certain Brazilian fruits were tried as flavors in ice cream are given. The juice of the passion flower fruit, known in Brazil as "maracuja," has a sour taste somewhat similar to orange juice; it was better suited for ices or sherbets than for ice cream. Juice of the cashew plant gave a pleasing, nut-like flavor which blended well with ice cream containing nut meats. The authors stress the desirability of using certain of these foreign fruits in ice cream. W.C.C.

45. **Nuts for Your Winter Ice Cream.** W. J. Caulfield and C. A. Iverson, Department of Dairy Industry, Iowa State College, Ames, Iowa. Ice Cream Field, *48*, 4: 84. Oct., 1946.

The use of nuts in ice cream enhances the nutritive value of ice cream, since nuts are good sources of fat, protein, vitamins (A, B, and G), and minerals (calcium, phosphorus, iron, and copper). Selection of nuts with good flavor, prevention of deterioration, and development of crispness as a result of proper roasting are important considerations, but the question of bacterial contamination of ice cream through the use of nut meats deserves more consideration than it has received in the past. Small manufacturers can use prepared nuts to advantage, whereas the large manufacturers ordinarily find it advantageous to roast the nuts in their own plants.

Directions are given for preparing the following nuts for use in ice cream: almonds, cashews, hazel nuts, peanuts, pecans, pinions or pignolias, pistachios, black walnuts and English walnuts. Roasting procedures cannot be used according to time and temperature charts. Frequent examination during roasting is necessary to assure that the nuts will be light brown in color throughout as well as brittle and crisp when finished. The nuts should be chopped after roasting and 6 to 8 lbs. of nuts and 1 oz. of salt mixed with 1 lb. of butter previously heated to 330° F. (160° C.). They may be used immediately or stored in a closed container in the hardening room. Use 3 to 5 lbs. of buttered nuts per 10 gal. of ice cream. W.C.C.

46. Some New Facts on Ice Cream in Super-Markets. R. W. Mueller, Associate Editor of Progressive Grocer. Ice Cream Field, *48*, 4: 32. Oct., 1946. (Reprinted from Progressive Grocer.)

A recent survey of super-markets in the Los Angeles area shows that ice cream occupies only 0.88% of store display facilities but represents 2.02% of total store volume and brings 4.7% of total dollar margin. This same survey shows that the rest of the frozen food line occupies 1.96% of total display space, represents 4.56% of total store volume and 7.14% of the total dollar margin. Ice cream represents 30% of the total frozen food department and equals 40% of the department's gross.

Ice cream sales now are mostly in pints and quarts. With larger frozen food storage compartments in household refrigerators, food merchants anticipate the sale of larger packages, such as half-gallon and gallon sizes.

W.C.C.

47. What to Do About Ice Cream Cabinets. Edward L. Koepenick, Ex-Secretary, National Conference of Ice Cream Industries. Ice Cream Field, *48*, 4: 112. Oct., 1946.

Certain unfair trade practices in connection with credits, repairs, advertising, etc., often used in the ice cream industry as a means of distributing ice cream cabinets and controlling ice cream sales, are discussed. Mention is made of the California Agricultural Code which prohibits these unfair trade practices. The author advocates Federal legislation designed to abolish such evils. W.C.C.

48. Layout of Market to Feature Ice Cream and Frozen Foods. Beman Fast, Store Planning and Market Fixture Division, Weber Showcase and Fixture Co., Inc., Los Angeles, Calif. Ice Cream Field, *48*, 4: 34. Oct., 1946.

The recently modernized Hollywood Ranch Market is described and the subsequent increase in sales noted. Self-service, glass-topped cabinets which replaced blind storage cabinets have resulted in a 60% increase in ice cream sales and have doubled sales in some departments. W.C.C.

49. Sale of Ice Cream by Weight. Anonymous. Ice Cream Trade Jour., *42,* 8: 32, 83. Aug., 1946.

The International Association of Ice Cream Manufacturers is opposed to the sale of ice cream by weight. Reasons for this stand are:

(1) The most expensive ingredient in ice cream, namely butterfat, is the lightest in weight.

(2) Although, proportionately, ice cream contains very much less air than does angel food cake, air in the proper quantities is just as important, and there is no more reason to sell ice cream by weight than there is to sell angel food cake by weight.

(3) In the proposed Food and Drug Administration standard for ice cream, the product must be agitated during freezing to avoid formation of a solid brick similar to a block of ice. The air in ice cream gives an insulating effect and makes ice cream melt more easily in the mouth.

(4) The most expensive ingredient of ice cream, butterfat, is lightest (7.76 lbs. per gal.), while its cheapest ingredient, invert sugar sirup, is heaviest (10 lbs. per gal.).

(5) A high-grade rich ice cream which would weigh not more than 4.6 lbs. or 4.7 lbs. per gal. may be made. A very cheap grade of ice cream, one poorer in almost all of the qualities that make a good ice cream, may be made to weigh 5.5 or 6 lbs. to the gallon.

(6) Most of the almost 200 different flavors which have been recorded for ice cream would have a different weight.

(7) More ice cream is sold at the soda fountain, in restaurants as individual servings or in ice cream cones than is sold to be taken home. It is entirely impractical to make sales of this kind by weight, even if it seemed desirable to do so. W.H.M.

MILK

50. Post War Milk Bottle. V. L. Hall, Glass Container Manufacturers Institute, New York, N. Y. Jour. Milk Technol., *9,* 6: 336–338. Nov.–Dec., 1946.

The square milk bottle gradually is being introduced into the dairy industry. In the dairy plant, the washing and handling of the square bottle is just as satisfactory as for the round bottle. A case of round bottles occupies 47.5% greater area than the square bottles; a 6-ft. household refrigerator will hold 12 square bottles in the area formerly occupied by 8 round bottles. Moreover, the square container permits the retail grocer to do a better job of packing groceries in bags and in shopping carts. H.H.W.

51. Strainer Pad Control of Milk Quality. C. B. A. Bryant, Johnson and Johnson Co., Chicago, Ill. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 199. 1945.

Observations on sources of sediment were made during field demonstrations on farms all over the United States. A clean cow and a clean milk can are essential. Milk-can covers are often in dusty place when not in use. About 40% of all water on the farm contains considerable sediment. Half of the farmers do not put the cotton disk in the strainer correctly. Cotton pads commonly distort during use. Single gauze-faced disks should be placed in the strainer with the cotton side up. Strainers are often jammed and disks loosen. The strainer should be rinsed with water before a new disk is inserted. Milking machine teat cups frequently draw up bedding.

New York State experiences have shown several things. Dairies use 8-inch cotton disks when 5.5- or 6-inch disks would do, and cost about half as much. Some 85% of the strainers were not good. A fluid milk plant was selected for demonstration. Each farmer demonstrated correctly how to put the 6-inch disk into the strainer. Good strainers were installed. Emphasis was placed upon sanitary methods rather than *cleaning milk.* Excellent farm cooperation was obtained. Original strainers and used cotton disks were obtained for exhibition. After proper farm instruction had been given and correct equipment used, results showed definite improvement in sanitary procedures and milk quality. A.C.D.

52. Laboratory Tests in the Control of Milk Supplies. Editorial. Amer. Jour. Pub. Health, *36,* 11: 1309–1310. 1946.

This editorial concerns the review article by Dr. A. H. Robertson in the same number of the American Journal of Public Health. In part, the editorial states: "Dr. Robertson's specific recommendations with regard to Plan B (the Connecticut State Department of Health milk program) deal with legal and administrative problems which are of purely local interest to the State of Connecticut and involve no changes in laboratory procedure. With respect to Plan A (the U. S. Public Health Service Ordinance and Code), on the other hand, he makes three important recommendations with regard to testing technique: the routine testing of all samples of allegedly pasteurized milk by the use of the phosphatase test and the coliform test; the routine examination of all samples of pasteurized milk by the microscopic count; and use of the microscopic count for samples of raw milk from sources which fail to comply, especially when field inspectional methods fail to disclose the cause for high counts or short reduction times.

This is not a matter to be settled by any one expert, as Dr. Robertson would admit; but he has marshalled evidence which makes an impressive case against sole reliance on the laboratory tests included in Plan A. There is a clear challenge here to the experts in this field—and particularly to the experts of the U. S. Public Health Service—to seek some common ground of agreement which can harmonize or combine the values of both Plan A and Plan B. We would urge that the procedure employed by water bacteriologists in their field be applied in this related area. This procedure involves the setting up of cooperative studies in which workers in various laboratories employ several alternative standardized procedures and compare results, with regard to the significance of results obtained in the examination of a considerable series of actual field samples and the time involved in each procedure. . . . Only by such a cooperative study, in which advocates of both Plan A and Plan B participate, can the solution be found of a difference of opinion which may otherwise place serious obstacles in the way of an effective system of milk control.'' M.W.Y.

53. **Laboratory Procedures in Sanitary Milk Control.** A. H. ROBERTSON, Director of State Food Laboratory, Department of Agriculture and Markets, Albany, N. Y. Amer. Jour. Pub. Health, *36,* 11: 1245–1259. 1946.

The author prepared this special review at the request of the Editorial Board. He concludes ''Under either the U. S. Public Health Service Ordinance and Code (Plan A) or under the Connecticut State Department of Health Program (Plan B), the fundamental objectives of maintaining the highest possible assurance of safety and continuous conformance to the standards for low count milk have not been as fully achieved as might be expected.

''Plan A would be improved by:

''1. Requiring the routine testing of all samples of allegedly pasteurized milk using the phosphatase test and the coliform test.

''2. Requiring a microscopic examination of all raw samples which fail to comply, especially when field inspectional methods fail to disclose the cause for high counts or short reduction times.

''3. Requiring routinely a microscopic examination of all pasteurized samples to determine whether or not high-count samples escape detection by the plate method.

''4. Permitting the use, where determinations have been checked periodically and found satisfactory, of routine plant reports on inspections and analyses of samples by licensed purchasing agencies in lieu of official inspections and analyses.

''Plan B would be improved by:

''1. Securing proper legislation fixing, or allowing the Dairy and Food Commissioner to fix by official order, standards for bacterial density in terms of results by methods which are to be used officially for the determination of compliance with the statute.

"2. Providing for more frequent routine inspections and examinations of samples, with prompt repeat inspections and repeat examinations of samples in cases of non-compliance until continuous conformance can be reasonably assured." M.W.Y.

54. **What the Dairy Industry Expects of the Sanitarian.** C. C. HADLEY, Indiana Dairy Products Assn., Inc. Milk Plant Monthly, *35*, 8: 24–25, 56. 1946.

A good milk sanitarian should be a teacher, diplomat, and administrator, thus exhibiting common sense, sound judgment, diplomacy and practicability, and radiating a pleasing personality through good health, energy, unquestioned integrity and enthusiasm for his work. A health sanitarian is expected to be an ambassador of good will for sanitation programs; to make proper contact with the manager's office prior to inspection; to use gentlemanly procedures in calling the management's attention to existing conditions; to be consistent month after month, not overlooking recommendations made on the previous visit; to use judgment on big versus little things; to refrain from peddling information from plant to plant concerning conditions; to be loyal to his superior; and to be well-trained for his job. Attention is called to a report appearing in the JOURNAL OF DAIRY SCIENCE, August, 1944, concerning courses which should aid in the training of a dairy sanitarian. G.M.T

MISCELLANEOUS

55. **Dairy Waste Saving and Disposal.** WILLIAM A. DEAN, JR., Bowman Dairy Co., Chicago; Chairman, Task Committee on Dairy Waste Disposal. Milk Indus. Found. Assoc. Bul., *39*, 2: 30–42. Dec., 1946.

The problem of waste is serious because litigation and punitive expense frequently result from overloading existing sewage disposal facilities. Systems of undue size may be required because of excessive quantities of waste. Without change in equipment, one plant reduced daily waste from a B.O.D. (biological oxygen demand) of 260.5 lbs. or a population equivalent of 1,580 to a B.O.D. of 73.4 lbs. or a population equivalent of only 450. Many causes of waste are listed. The following recommendations are made: drip savers; pre-rinses; electronic level controls on tanks and troughs subject to overflow; improved maintenance of pumps, fittings, valves, etc.; elimination of foam in separation; adequate storage tanks for whey, buttermilk, and skim milk; standby pumps where needed to handle these products if regular pumps fail; special entrainment separators and other controls on vacuum pans. Special emphasis is placed upon preventing dilution of wash waters and necessary wastes with condenser and clear waters.

Every plant seemingly in need of additional waste treatment facilities should make sure that losses are reduced to a minimum. Use of continuous proportionate sampling device and determinations of turbidity and B.O.D. are recommended. E.F.G.

56. The Effect of Dairy Factory Drainage Upon the Quality of Streams in Taranaki. P. O. VEALE, Taranaki Service Laboratories, New Zealand. New Zeal. Jour. Sci. and Technol., *27*B, 4: 282–301. Jan., 1946.

With reasonable dilution available, the discharge of dairy plant wastes into a well-oxygenated stream had only a temporary effect upon the quality of the water. The greater the dilution, the shorter was the distance downstream to where effects were no longer evident. W.C.F.

57. The Oregon Program of Licensing Cheese-Makers, Butter-Makers and Pasteurizer Operators. G. H. WILSTER, Oregon State College, Corvallis, Oregon. Jour. Milk Technol., *9*, 6: 317–321, 328. Nov.–Dec., 1946.

The licensing of cheese-makers, butter-makers, and pasteurizer operators is an important step in Oregon's dairy products quality-improvement program. The compulsory milk and cream grading law enacted in 1937 provides for the grading of these products when received at factories and creameries, licensing of persons who are doing the grading, and payment for the milk and cream in accordance with quality. Inferior quality of milk and cream must be denatured by adding red coloring matter and must be tagged, and returned to place of origin. In 1939, an amendment requiring the issuance of licenses upon passing of an examination for butter- and cheese-makers made the act much more effective.

A new law, passed in 1945, governs the pasteurization of milk and milk products and licensing of pasteurizer operators. The ultimate goal of these standards is: To raise the general standard of proficiency of the Oregon butter-makers and cheese-makers, to manufacture the highest-quality cheese and butter of correct composition, to increase the demand for Oregon cheese and butter in out-of-state markets, and to increase the return to the Oregon dairy farmers. H.H.W.

58. Insect Control in Dairy Plants. GEORGE E. GOULD, Purdue University. Milk Plant Monthly, *35*, 7: 38, 54–55. 1946.

Control of dairy farm and dairy plant insects is essential to the manufacture of quality dairy products. Insect control is linked closely with plant sanitation and is actually a part of that program. Insects are attracted to dairy plants because of milk and milk products, although some insects are attracted because of lights. The first and most important step in fly control is sanitation. Thorough daily cleaning of all equipment used is imperative. Daily burning and disposal of refuse is essential. Sixteen-mesh screening will check entry of flies, mosquitoes, gnats, and other small insects into the plant. The use of DDT should not be considered as the only necessary control measure, but as supplementary to sanitation. Specific directions are given for applying DDT. Descriptions are given of the various roaches infesting dairy plants. Sodium fluoride alone, or diluted with 25% pyrethrum powder, is a standard roach powder. Sprays have never been entirely successful in roach control. Sanitation is an important part of roach control. G.M.T.

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United States Department of Agriculture

ABSTRACTS OF LITERATURE

BACTERIOLOGY

59. **Comparison of Resazurin Test with Methylene Blue.** G. OKULITCH, R. MILLARD, AND O. FLEMING. Canad. Dairy and Ice Cream Jour., *25,* 11: 35. Nov., 1946.

Milk can be graded effectively by means of the resazurin test using a single color standard, mauve pink no. 12 or Munsell notation P.R.P. 7/8, and making readings at 1 and 3 hr. The resazurin test and the methylene blue test agree well in selecting poor-quality raw milk. The correlation of resazurin and methylene blue is approximately 80%. Both the standard plate count and the direct microscopic count support the resazurin test in more cases than they do the methylene blue test. A high percentage of samples that test 3 hr. or more with resazurin have counts of less than 200,000 per ml. by the plate method and less than 100,000 by the direct microscopic procedure. A resazurin mauve pink of 3 hr. or more represents as good-quality milk as a 6.5-hr. methylene blue test. A greater number of physiologically or pathologically abnormal milks may be detected by the resazurin test as compared to the methylene blue test. By using the resazurin test a greater saving of time is effected. H.P.

60. **Thermal Death Range of Bacteria in Milk.** F. W. GILCREAS AND J. E. O'BRIEN, Research Laboratories, New York State Department of Health, Albany, N. Y. Jour. Milk Technol., *9,* 5: 269–272. Sept. and Oct., 1946.

See Abs. 21, Jour. Dairy Sci., *30,* 2: A14. Feb., 1947.

CHEESE

61. **Factors Influencing Acid Production by Cheese Cultures. I. Effect of Cooking Temperatures on Acid Production in the Manufacture of Cheddar Cheese.** F. J. BABEL, Iowa Agr. Expt. Sta., Ames. Natl. Butter and Cheese Jour., *38,* 1: 34. Jan., 1947.

An abbreviated form of the original publication in Jour. Dairy Sci., *29,* 9: 589. Sept., 1946. W.V.P.

62. **Making Process Cheese in Small Plants.** C. R. BARKER, Oak Park, Ill. Natl. Butter and Cheese Jour., *37,* 12: 90. Dec., 1946.

Cheese that has been aged for 30 days at 60° F. without paraffin can be used to make a satisfactory processed cheese. Water lost in curing is replaced during processing. In blending, there should be 50% of cheese "on the sweet side" and 50% "on the acid side". Cooking this blend of cheese with water in the jacket of the cooker at a pressure of 10 lbs. and a temperature of 239° F. is recommended. (No warning is given concerning ability of the cooker to stand the pressure.) W.V.P.

63. Practical Suggestions on the Manufacture of Process Cheese. C. R. Barker, Oak Park, Ill. Natl. Butter and Cheese Jour., *38,* 1: 42. Jan., 1947.

Cheese made in a single factory can be processed successfully if some is made "on the acid side" and some "on the sweet side". Vat drippings of 0.9% before salting give cheese of the acid type. Such cheese should constitute 30–40% of each batch, while the remainder should be "sweet" from curd with vat drippings of 0.6% at salting. Tough, over-cooked Cheddar is not desirable. The usual cleaning and cooking operations are described very briefly. A diagram illustrates the flow of material through the operations of curing, grading, blending, cleaning, grinding, cooking, packaging, cooling, and shipping. W.V.P.

CHEMISTRY

64. Some Chemical Changes Produced in Milk by High Temperature Heat Treatment. Ira A. Gould, Jr., Dairy Dept., University of Maryland. Milk Plant Monthly, *35,* 9: 70–71. Sept., 1946.

The author reviews brifly results of his research in this field during the past 12 years, pointing out the relationships between the boiled or cooked flavor of milk and some chemical changes bringing out these flavors. The cooked flavor was caused by the formation of sulfhydryl compounds, usually accompanied by the evolution of hydrogen sulfide gas at temperatures of 168.8–172.4° F. These chemical changes become more pronounced as the temperature increases to boiling. Serum proteins were undoubtedly the principal contributors to the formation of the heat-labile sulfides. Extremely small quantities of metallic salts of mercury, silver, ferric iron, and copper influence sulfide liberation. Additions of small percentages of sucrose, glucose, and lactose lower sulfide liberation. On the other hand, cysteine hydrochloride, sodium cyanide, sodium sulfite, and ethyl alcohol favor sulfide liberation. Prolonged heating of milk at high temperatures produced further changes in sulfide liberation, which is associated with a caramel flavor and a brown color. High heat treatment, particularly under pressure, produced appreciable increases in titratable acidity and destruction of a considerable portion of the lactose. This destruction is favored by the presence of sodium citrate or disodium phosphate. Salts of milk play a significant rôle in the amount of titratable acidity increase. Less than 10% of the acidity was due to lactic acid and 50 to 60% of the heat-produced acidity was found to be formic acid. This work shows the complexity of the changes occurring in milk at high temperature under various conditions. G.M.T.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

65. The Future of Dry Milk. STEPHEN O'DEA, U. S. Dept. of Agr. Natl. Butter and Cheese Jour., *38*, 1: 80. Jan., 1947.

Increased use of dry milk in some areas through the school lunch program would improve the health of children. Government-owned facilities for producing dehydrated dairy products are located in five states. "It is not feasible to close down the drying facilities and shut off these outlets for farmers' whole milk." Some expansion in U. S. production of nonfat milk solids seems likely. "More thinking needs to be done about the development of markets for nonfat dry milk than for any other manufactured dairy product." W.V.P.

66. The Manufacture and Use of Condensed Cheese Whey and Crude Whey Protein. B. H. WEBB AND C. F. HUFNAGEL, U. S. Dept. of Agr., Washington, D. C. Natl. Butter and Cheese Jour., *37*, 12: 34. Dec., 1946.

Cheese whey can be condensed to 1/10th its volume for 1 to 1.8¢ per lb. of finished product. For human food the whey is pasteurized as soon as it is removed from the cheese. It can be condensed with or without sugar. Unsweetened whey is concentrated to 65–70% total solids. Crystallization of the lactose is controlled by prompt condensing in a clean pan, drawing concentrate as a clear sirup, cooling rapidly at 90° F., seeding with lactose crystals, and stirring. Plain condensed whey with a pH of 4.5 or less can be packed in air-tight barrels and kept for several months at cool temperatures. Sweetened condensed whey (see Jour. Dairy Sci., *21:* 305–314. 1938.) is made by separating, pasteurizing, adding sugar in amounts to equal the weight of whey solids, and condensing to 76% total solids. This concentration at 122° F. gives 1.360 sp. gr. (38.4° Be). The concentrate is cooled to 95° F., seeded, stirred slowly for 1 to 3 hr., and packed in barrels or cans. Refrigerated storage is not required. Whey protein for pharmaceutical uses is separated from whey by heat and acid. The curd is washed with water, drained, pressed, and preserved by drying or freezing. Albumin curd can be dried rapidly at 110–120° F. in a tunnel drier. Some pharmaceutical companies will furnish specifications of purity for albumin powder. The residue can be concentrated for feed or milk sugar. Equipment for sugar manufacture can be supported only by large operations.

Successful commercial uses for condensed whey in food products are limited at present to confections, bakery goods, and cheese foods. Characteristic flavor and the insolubility of its lactose make whey inferior to skim milk for food purposes. It has high nutritive value and low cost. Formulas are given for whey candy and cookies. Methods of using whey products in bread, sweet baked goods, cheese foods and cream soups are suggested. W.V.P.

67. A Method for Producing a Dairy Spread. K. G. WECKEL, Dept. of Dairy Industry, University of Wisconsin, Madison, Wis. Milk Plant Monthly, *35,* 9: 24–25. Sept., 1946.

A method for producing a dairy spread containing 28% butterfat, 19% milk solids-not-fat, with and without added vitamins, is described and illustrated. The ingredients are whole milk powder, cream, buttermilk, lactic acid, salt, vitamins A and D, and starter distillate. After pasteurizing, the spread is homogenized at a pressure which will give the greatest plasticity without graininess (usually varying from 1,500 to 2,500 lbs.). The product is packaged hot, preferably in glass containers of the cottage-cheese jar type. The product sets upon cooling and may be kept 1 or 2 weeks, similar to any soured milk product. G.M.T.

68. Milk Sugar. GERTRUDE G. FOELSCH AND HARRY C. TRELOGAN, Production and Marketing Administration, Washington, D. C. Milk Plant Monthly, *35,* 11: 40–41, 48. Nov., 1946.

Renewed interest is being manifest in milk sugar because it is playing an important rôle in penicillin production. A program was inaugurated in 1943 to increase milk sugar production from cheese whey. Manufacturers find partial recovery of crude milk sugar more profitable, using the remaining mother liquor to produce poultry feed. Three grades of commercial milk sugar—crude, technical, and refined—are produced. Emphasis today is being placed on production of the crude form for use in the manufacture of penicillin, although formerly milk sugar was consumed largely in infant foods. G.M.T.

FEEDS AND FEEDING

69. Vitamin A Requirements in Calves, Part I. J. M. LEWIS AND L. T. WILSON. New York University College of Medicine, New York City, and The Walker Gordon Laboratories, Plainsboro, N. J. Cert. Milk, *21,* 244: 5. Aug., 1946; Part II, Cert. Milk, *21,* 245: 9. Sept. and Oct., 1946.

Six groups of four calves each were fed various levels of vitamin A, ranging from 32 to 1,024 USP units per kg. of body weight per day. Data were obtained on rate of growth, blood levels of vitamin A, and liver stor-

age. Results indicate that 32 units per kg. of body weight apparently satisfies the minimum requirements. Maximum growth was obtained on an intake of 64 USP units per kg. of body weight. The concentration of vitamin A in the blood was proportional to the intake until 512 units were given, at which level maximal blood concentrations were obtained. In general, liver stores were quite low for calves receiving 32, 64, and 128 units per kg.; moderate amounts of vitamin A were found in the livers of those fed 256 to 512 units, and larger amounts in the group fed 1,024 units. From the standpoint of both growth and liver storage, the daily intake of vitamin A for young calves should be about 250 units per kg. of body weight or 11,000 units per 100 lbs. of liveweight. Thus, the vitamin A requirements in calves are of the same order of magnitude as in young rats and in infants.
W.S.M.

FOOD VALUE OF DAIRY PRODUCTS

70. **Between Meal Milk Drinks Beneficial for Children.** National Dairy Council. Canad. Dairy and Ice Cream Jour., *25,* 11: 78. Nov., 1946.

No adverse effect on the appetite or well being of children ranging in age from 3 to 14 years when fed milk 1 hr. before meals was observed. Tests on 59 children revealed an average stomach-emptying time of 118 min., representing a range of 50 to 170 min. The contributions of two levels of milk (44 and 63 oz.) to the total daily nutrient intake were, respectively: 40 and 49% for calories, 35 and 45% for protein, 85 and 91% for calcium, 30 and 41% for vitamin A, 55 and 65% for thiamine, and 80 and 87% for riboflavin. Each 7 oz. serving of milk contributed approximately 5% of the total caloric intake. H.P.

ICE CREAM

71. **Good Methods of Manufacture for Dry Ice Cream Mix.** S. T. Coulter. Canad. Dairy and Ice Cream Jour., *25,* 11: 61. Nov., 1946.

See Abs. 42, Jour. Dairy Sci., *30,* 2: A20. Feb., 1947.

MILK

72. **Quality Milk from Cow to Milk Plant.** C. B. A. Bryant, Johnson & Johnson, Chicago, Ill. Milk Plant Monthly, *35,* 12: 26–27, 52–53. Dec., 1946.

Emphasis is placed upon keeping sediment out of milk, as sediment is one of the common causes of milk rejections. Despite care in cleanliness during the production of milk, sediment gets into milk from loosely covered cans or from nonrinsed cans prior to use. Wind-blown dust is often the cause of sediment in milk. Lids of cans, as well as the can itself, should be protected from dust. Rinse water on the farm may be the source of sediment. Displaying the sediment disc and pointing out the common causes for sediment in milk are aids in keeping the milk clean. The responsibility for clean milk rests not only with the producer but also with the hauler and processor. G.M.T.

73. Evaluation of Factors when Processing Homogenized Milk. E. M. GIBERSON. Canad. Dairy and Ice Cream Jour., *25*, 11: 52. Nov., 1946.

Homogenized milk always must be pasteurized, clarified, and processed rapidly and at proper temperatures and pressures. The advantages of homogenized milk are that it produces uniformity of appearance, pouring characteristics, flavor, and color. Before a plant operator purchases equipment and starts processing homogenized milk, he should consider type of pasteurization to be employed, clarification and filtration methods, capacity of various pieces of equipment, and advantages and disadvantages of each. The equipment needed for homogenizing milk requires a considerable capital investment in milk processing equipment.

74. Flavors in Milk Influenced by Pastures and Cattle Feeds. JACK BAILEY. Canad. Dairy and Ice Cream Jour., *25*, 11: 59. Nov., 1946.

The problem of preventing the flavors of feed from getting into the milk depends on the time of feeding. It is generally agreed that feed flavor is no longer evident in the milk 5 hr. after feeding. The cows should be kept in an atmosphere free from undesirable odors before milking. If the flavor is due to silage or barn feeds, it is advisable to feed after milking. If the flavor is due to pasture weeds or feeds, it would seem best to bring the cows off the offending pastures preferably 5 hr. and at least 2 hr. before milking. The development of a cowy, old, stale, and then rancid flavor in milk is due to lipase action. This activity is aggravated by shaking whole warm raw milk and by milking cows in advanced stages of lactation. The mixing of milk likely to become rancid with four or more parts of normal milk always will prevent rancidity. In pasteurized milk, oxidized flavor may be caused by absence of bacteria. Metallic, fishy, oily, and tallowy flavors, which are common in pasteurized milk, are caused by dissolved oxygen, copper contamination, oxidase enzyme, and exposure of milk to direct sunlight. H.P.

75. Control of Milk Watering. Paul Corash, Dept. of Health, New York, N. Y. Milk Plant Monthly, *35*, 10: 90, 92–93, 96. Oct., 1946.

A study of the detection of milk watering by means of the Hortvet cryoscope, an instrument essentially adaptable to laboratory use but chosen for field use, indicated a relatively large percentage of the samples of producers' milk examined had been watered. The lactometer was used to screen out suspicious cases. Calculating the solids-not-fat content of milk furnished the basis also for judging whether or not a sample of milk should be tested with the cryoscope. Importance is placed on securing relatively fresh samples in order to secure correct cryoscope values. Other methods, such as the copper-serum method and the chemical determination of fat, total solids, and ash on deck samples, also may be used in determining watering, but are considered less accurate than the cryoscope method.

G.M.T.

MISCELLANEOUS

76. Vacreation of Cream, Milk, and Ice Cream Mix and Condensing Milk with the Vacreator. G. H. Wilster, Oregon State College, Corvallis, Ore. Milk Plant Monthly, *35*, 11: 28–32. Nov., 1946.

The vacreator is illustrated and the steps involved in its operation are described fully, the process consisting briefly in heating the milk product to approximately 200° F. and passing it through a series of chambers with increasing vacua until the product is removed at a markedly lower temperature. The process removes gases and off-odors present in the product; consequently the finished product will be free of off-odors. Butter manufactured from cream by the evacuation process had a higher score than that not treated. Ice cream of an excellent quality was produced from vacreated ice cream mix with which vacreated condensed milk was used.

G.M.T.

77. Recent Developments in Dairy Manufacturing Through Research. G. H. Wilster. Canad. Dairy and Ice Cream Jour., *25*, 9: 34; 10: 54. Sept. and Oct., 1946.

Some of the recent developments in dairy manufacturing based on research are: (1) the continuous high-temperature short-time pasteurization of milk in a totally enclosed apparatus using clarification, homogenization, and an enclosed cooler; (2) single service containers for milk; (3) sterilized cream; (4) sterilized milk; (5) the use of the vacreator in the dairy industry; (6) metal churn for buttermaking; (7) dry butterfat or butteroil; (8) continuous butter churns; (9) curing cheese in valve-vented cans; (10) packaging rindless cheese in moisture-proof sheets; (11) Army cheese spread; (12) dry ice cream mix; (13) dry whole milk; (14) dry cream mix for whipping by aeration; (15) sweetened dry nonfat milk solids; (16) frozen concentrated milk; (17) use of cheese whey for candy, soups, puddings, plastics, penicillin; and (18) dairy spreads. H.P.

78. Flocculation and Sterilization Without the Use of Chemicals. W. R. MARSHALL. Canad. Dairy and Ice Cream Jour., *25,* 10: 27. Oct., 1946.

Water can be purified and sterilized in any flow-rate gravity or pressure system by the use of aluminum electrodes as a flocculator and silver as a sterilizer. The flocculating unit operates on a D.C. current of 6 volts, 3 amperes, which supplies the current for the flocculating electrodes. Unlike ordinary sand filters which depend on chemicals alone to produce the floc, the system is not affected by the lower temperature of the water. Immediate sterilization is obtained since the coagulant produced by the aluminum electrode holds the silver in an ionized form, immediately attracting and destroying bacteria. The amount of silver ions needed to sterilize water is in the neighborhood of one part in ten million. With this apparatus, which consists of a sand filter tank with rapid flocculators to remove the dirt and the silver electrode to destroy the bacteria, use of any chemicals is not necessary. H.P.

79. The Use and Abuse of Wetting Agents as Applied to the Cleaning of Milking Machines. HARLOW L. PENDLETON, Massachusetts Department of Agriculture. Milk Plant Monthly, *35,* 12: 30–32, 70, 72. Dec., 1946.

Experimental data indicated that the flush washing of milking machines using wetting agents was as effective in reducing bacteria counts of milking machine units as brush washing. Also, the cold water prerinsing of milking machines could be eliminated for all practical purposes. After 7 days' treatment, low counts were obtained on milking machines, with no appreciable increase after 14 days. Milkstone deposits were insignificant at the end of 7 days. The flush-washing method of cleaning milking machines, employing wetting agents, was not advocated as a cure-all for cleaning. The process should be used with caution by the careless milk producer. However, the careful operator can clean the machine units with less time and labor when using this method than by brush washing. Until more is known of various detergents and cleaning qualities of wetting agent compounds, intermittent use of brush washing of milking machines must be recommended. Carefully selected detergents with wetting properties, intelligently used, should prove a great boon to the dairy farmer.

G.M.T.

80. Labor-saving Methods and Materials for Dairy Plant Cleaning. D. H. Jacobsen, Cherry-Burrell Corporation, Chicago, Ill. Milk Plant Monthly, *35*, 11: 24–27, 36. Nov., 1946.

Advancements in machinery design and building materials and layouts make imperative higher standards of plant sanitation despite higher labor costs. Choice of products for cleaning in milk processing plants often is made on bases of wetting properties, water-softening powers, costs, and availability. Properties desired in a good cleaner are: (1) quick and complete solubility, (2) non-corrosive on metal surfaces, (3) complete water-softening or water-conditioning power, (4) good wetting or penetrating action, (5) emulsifying action on fat, (6) dissolving action on milk solids, (7) deflocculating, dispersing, or suspending action, (8) good rinsing properties, (9) germicidal action, (10) economy in use. Straight alkalies, acids, or wetting agents alone do not meet the requirements of a good cleaner. Likewise, a universal cleaner does not exist, since it is not practical to use one cleaning agent on all jobs. Hardness of water plays an important rôle in the efficiency of the cleaner. Phosphates improve the action of all dairy cleaners in hard water. Wetting agents are generally the most expensive component of dairy cleaners. Proper lighting, adequate ventilation, use of pipe wash tanks, storage racks, plant layouts, and machines influence the speed and effectiveness of cleaning. Circulating cleaning solutions and spray systems offer promise in reducing hand labor in dairy plants. Both acid- and alkaline-type circulating cleaning solutions are used in enclosed systems, such as plate heat exchange systems. Spray systems have decided advantage in dairy plant cleaning for large tanks or vats and surface coolers. Portable cleaning units offer a possibility in facilitating cleaning operations. G.M.T.

81. Cleaning Electrical Windings. D. L. Gibson, Westinghouse Electric & Manufacturing Co., East Pittsburgh, Pa. Natl. Butter and Cheese Jour., *38*, 1: 40. Jan., 1947.

Methods of cleaning motors are varied to suit the type of cleaning job required, *i.e.*, removal of grease, softening of varnish for rewinding, removal of effects of exposure to chemicals or flood waters. Standard methods of cleaning include rubbing with lintless cloths soaked in selected petroleum distillates or carbon tetrachloride, use of vacuum cleaning apparatus, spraying or immersing in solvents, and washing with water. It is not advocated that facilities be kept available for such diverse methods, but rather that the job of cleaning be analyzed carefully to save money, time, and labor. W.V.P.

82. Determination of Refrigerant Pipe Size. H. M. Hendrickson, Preston Construction Co., Division of Safeway Stores, Oakland, Calif. Refrig. Engin., *52,* 4: 317–325. Oct., 1946.

The author emphasizes that pressure drop, and not velocity, is normally the governing factor to be employed in sizing refrigerant piping. The chief consideration of refrigerant velocities is to keep them low enough to eliminate excessive noise in the lines, and not so low as to interfere with proper oil return. The author presents tables and charts for the more common refrigerants, with the greatest emphasis on the two most popular (Freon 12 and ammonia) giving the best available information on pressure drop to facilitate the determination of the proper size of refrigerant mains.

L.M.D.

83. Motor Transport Refrigeration. Part I—A Modern Refrigerating Unit. Henry O. Kirkpatrick, Advance Manufacturing, Inc., Detroit, Mich. Refrig. Engin., *52,* 6: 521–524. Dec., 1946.

The modern trend in truck refrigeration is toward the self-contained or package-type unit, comprising a compact installation of gasoline engine with starter, compressor, condenser, and receiver fastened on a rigid frame. Above the frame is an insulated platfrom upon which is located the evaporator, heat exchanger, expansion valve, and oscillating fan. The lower portion is closed from the truck body with an insulated bulkhead. Access to the lower compartment of the unit is had by two doors in the front wall of the trunk through which minor service can be rendered or control valves operated. Major servicing can be done from the interior by removal of the bulkhead. Replacement is readily made with a new unit if shop tear-down is required. In this modern unit provision is made to use the reverse cycle employing the refrigerating unit as a heat pump, the evaporator functioning as a condenser and the condenser as the evaporator. This versatility enables the operator to maintain a truck temperature of 0° F. against 80° F. outside or to maintain a temperature of 68° F. inside against that of − 15° F. outside if protection of that sort is needed. The frame installation of the mechanical components of the unit overcomes the road vibration encountered in truck transportation. To protect against excess pressures during shut down, hand valves (six in case of the reverse cycle unit) all are closed completely, isolating the compressor from the rest of the system. Automatic regulation of suction pressure to 30 psi prevents overload during pull-down of truck body temperature from 100° F. to 35° F. design temperature. Another trend will be that of units of 3 to 5 ton refrigeration capacity, capable of maintaining − 10° F. with 3 in. insulation, instead of the present 1 to 2 ton units used to maintain 32° F. with 3 in. insulation or 10° F. in trucks insulated with 6 in. of insulation. This is because the larger capacity unit takes up little more loading space and together with 3 in. insulation weighs much less than the smaller unit with 6 in. insulation. Also, there is a much greater gain in pay load space, resulting in a lower operating cost per ton hauled. L.M.D.

84. The Use of Silica Aerogel as a Thermal Insulation. F. FAXON OGDEN AND JOHN F. WHITE, Monsanto Chemical Co., Merrimac Division. Refrig. Engin., *53*, 5: 411–414. Nov., 1946.

Silica aerogel is a light, free flowing, voluminous solid having a density of about 7.0 lbs. per cu. ft., approximately 94% of its volume being air. It has a *k* factor about 10% less than the theoretical value for still air. This is explained on the basis of the pore diameter in the aerogel being about 250 Ångstroms, which is less than the mean free path of the molecules in free air. This causes a reduction in the molecular movement of the air enclosed in the pore spaces and lowers the conductivity of that air below normal. For a mean temperature of 0° F., *k* is given as 0.13 and for - 50° F., as 0.115. Moisture-vapor imperviousness is practically 100% for silica aerogel, but it must be protected against liquid water, for when over 15% by weight is absorbed, the aerogel structure collapses and cannot be restored to its original state. Equilibrium in natural settling is reached after about 5 hr. Mechanical vibration will hasten settling, and by this means speed up in filling cabinet spaces may be obtained. When silica aerogel is subjected to mechanical load, there is initially a relatively large decrease in volume due to closer packing of the individual particles. This is not recovered upon release of pressure. Fire hazard is nonexistent because the material for insulation is heat-treated to remove 7 to 10% volatile matter of inflammable nature. This material is not silicotic, but a respirator mask is advised because of the dust. Because its *k* factor is about one-half that of materials used for freezer cabinet insulation, a great increase in volume of storage space may be realized without increasing external dimensions, while in the ordinary refrigerator an increase in capacity of between 80 and 90% may be realized. When used in normal spaces designed for other insulating materials, the low conductivity of silica aerogel reduces refrigeration unit operation 40 to 50%. Research is being continued and has already resulted in a product of 3.5 to 4.0 lbs. per cu. ft., retaining all the favorable properties of the original aerogel. L.M.D.

85. Swedish Insulant Offers Useful Properties. THORE M. ELFVING, Stockholm, Sweden. Refrig. Engin., *52*, 4: 311–313. Oct., 1946.

Details are given of the physical properties of Isoflex, a thermal insulant of air layer type made from thin corrugated foils of cellulose acetate. The foils have a thickness of approximately 0.0015 in. These thin foils are joined with their corrugations at right angles, making a punctiform contact between them. The several foils are joined into slabs of varying thicknesses without adhesive material, being "welded" together by fusing the cellulose acetate at the points of contact. The slabs are 24 in. by 24 in. In order to impart black body property to the cellulose acetate foil, which is much thinner than 0.004 in., an opacifier is added to the cellulose acetate before forming into the foil sheets. This insulant is very light, weighing only 0.67 to 0.8 lb. per cu. ft. It can be cut readily to fit into irregular spaces.

In applying Isoflex, the only requirement is that the slabs fit tightly. The insulating layer should be slightly thicker than the space allowed for it, so that the slabs are compressed slightly. Its flexibility lends it admirably to the insulation of tank trucks for transportation of vegetable oils, milk, and other goods. The thermal conductivity of Isoflex is not given.

L.M.D.

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

86. **Milk Marketing under Federal Control.** CARL MCFARLAND, Formerly Assistant Attorney General of the United States. 205 pages. $7.50. Milk Indus. Found., Washington, D. C. 1946.

This book deals in a technical legal manner mainly with regulation conducted under the Agricultural Marketing Agreement Act of 1937. The growth in the last 50 years of legislation by administrative directive rather than by statute is commented upon. Most dairy laws in the past have consisted of rather narrow and specific governing statutes with provision for judicial enforcement. Broad administrative powers are given in the Food, Drug and Cosmetic Act of 1938 and still broader ones in the Agricultural Marketing Agreement Act of 1937. The official code carries the last four sections of the 1937 act under the chapter title "Agricultural Marketing Agreement Act" and the remainder under the "Agricultural Adjustment Act of 1933".

The essential substance of the Marketing Act is the fixing of minimum producer prices to be paid and prescribing how they are to be paid by handlers. In discussing detailed procedure and administration, the statement is made that the Marketing Act prescribes what is in some essential respects the most complicated procedural system on the Federal statute books. Detailed steps are outlined covering mediation and arbitration, the marketing agreement and order procedure, administrative and judicial review of orders, enforcement provisions and methods, and, finally, organization and personnel. Responsibility for the administration of the act rests with the Secretary of Agriculture. The final chapter gives a summary of typical marketing order provisions and a review of the issues which underlie them.

Appendices include a transcript of the Act, rules of practice and procedure, and marketing order references to the Federal Register for 49 different markets. E.F.G.

87. **Bovine Mastitis.** A symposium edited by RALPH B. LITTLE AND WAYNE N. PLASTRIDGE. 546 pages, illustrated. $7.00. 1946. McGraw-Hill Company, Inc., 330 West 42nd St., New York 18, N. Y.

This book is a thorough and critical treatment of the various aspects of this widespread and costly disease of dairy cattle. The editors and publisher are to be commended for their method of having the various sections or chapters written by recognized authorities in the field. The subject is covered in a broad and critical sense and wide differences of opinion recognized. The anatomy of the udder, the physiology of milk secretion, the pathology, diagnosis, bacteriological classification, transmission, treatment, control and public health significance of mastitis are among the subjects covered. An appendix giving details of tests, laboratory techniques, and methods is a useful addendum. Although this book is prepared in the form of a symposium, it should prove to be a useful text for students and investigators in the field and a reference work for veterinarians, dairymen, public health officials, and milk sanitarians. T.S.S.

88. The Problem of Fertility. Edited by EARL T. ENGLE. 254 pages. $3.75. 1947. Princeton University Press, Princeton, N. J.

This publication consists of a series of 16 papers presented at the Conference on Fertility sponsored by the National Committee on Maternal Health. These papers cover fields of active interest and investigation relating to the problem of fertility in man and animals. The inclusion of the discussions which followed the presentation of each paper provides additional interesting information and points of view.

The following subjects are presented: Patterns of Estrous Cycles, by S. A. Asdell; Ovulation in Sheep and Goats, by R. W. Phillips, R. M. Fraps, and A. H. Frank; Induction of Ovulation and Subsequent Fertility in Domestic Animals, by L. E. Casida; the Induction of Ovulation in Domestic Animals, by John Hammond; the Ovary at the Time of Ovulation, by George W. Corner; Hormonal Control of Ovulation, by H. H. Cole; Cervical Mucus and the Menstrual Cycle, by W. T. Pommerenke and Ellenmae Viergiver; Spermatozoa and Cervical Mucus, by A. R. Abarbanel; Glycolosis, Livability, and Fertility of Bovine Spermatozoa, by G. W. Salisbury; Metabolism and Motility of Human Spermatozoa, by John Macleod; Fertilizing Capacity of Rabbit Spermatozoa, by M. C. Chang; Biology of Equine Spermatozoa, by Victor R. Berliner; Artificial Insemination of Dairy Cattle, by J. W. Bartlett; The Cervix Uteri in Sterile Matings, by Fred A. Simmons; The Effect of Synthetic Thyroprotein on Sterility in Bulls, by E. P. Reineke; and Methods of Determining the Time of Ovulation in Domestic Animals, by John Hammond.

This book should prove of special interest and value to those engaged in the various phases of artificial insemination work. The discussions are well supported by experimental data and illustrations. T.S.S.

BACTERIOLOGY

89. The Growth of Coliform Bacteria in Pasteurized Milk Stored at Refrigerated Temperatures. A. C. DAHLBERG, Dept. of Dairy Ind., Cornell University, Ithaca, N. Y. Dairy Indus. Found. Assoc. Bull., *39*, 4: 86–95. Jan. 10, 1947.

Results previously published by the author (Jour. Dairy Sci., *29*: 651–

655, 1946) are given in detailed tables which show that coliform bacteria increase more rapidly in numbers than other bacteria in pasteurized milk held at refrigeration temperatures. Coliform bacteria were present in the majority of freshly pasteurized milk in one quart volumes. After storage at 45–50° F. and 55–60° F., the coliform bacteria constituted significant percentages of the total bacterial content. Since coliform bacteria in themselves are not a public health problem unless present in excessive numbers, a standard for condemnation might well be one that can be met by the best sanitary plants in the summer when counts are naturally the highest. No numerical standard is proposed by the author. E.F.G.

90. **Coliform Bacteria Problem and Its Control.** A. C. DAHLBERG, Dept. of Dairy Ind., Cornell University, Ithaca, N. Y. Milk Dealer, *36*, 4: 130–133. Jan., 1947.

See preceding abstract.

91. **Coliform Organisms in Pasteurized Milk.** C. J. BABCOCK, Market Milk Specialist, B.A.I., Agr. Res. Admin., U. S. Dept. Agr., Washington, D. C. Milk Indus. Found. Assoc. Bul., *39*, 4: 78–85. Jan. 10, 1947.

During the war the Medical Department of the Army made extensive application of the coliform test at Army installations throughout the country as an index to the sanitary conditions under which pasteurized milk is handled. Milk to be tested was inoculated into five tubes. If three or more of the tubes showed fermentation, the sample was considered positive for coliform organisms. Army installations adopting this method obtained very satisfactory results. A positive test may be due to improper pasteurization, heat-resistant organisms, excessive contamination of raw milk, or growth after pasteurization. There was no correlation between the results of the coliform test and the standard plate count. Positive and negative coliform results were obtained on both low and high count milk. Whenever coliform organisms are found in the milk delivered to the consumer, the condition almost invariably can be corrected by a thorough cleanup of the plant equipment. Thus the test is an ideal means of checking the cleanup operations in a plant. E.F.G.

92. **The Viability of Dried Skim-Milk Cultures of *Lactobacillus bulgaricus* as Affected by the Temperature of Reconstitution.** MARVIN L. SPECK AND ROBERT P. MYERS, National Dairy Products Corp., Baltimore, Md. Jour. Bact., *52*: 657. Dec., 1946.

"In spray-dried skim-milk cultures of *Lactobacillus bulgaricus* a large number of cells that failed to grow when the temperature of the reconstituting fluid was 21 to 25° C. were activated sufficiently to produce normal growth when the temperature of the reconstituting fluid was 37 to 50° C. When the culture was reconstituted at 21 to 25° C. and warmed to 50° C., the activating effect of the heat was not obtained.

"The increase in the colony count resulting from reconstitution at 50° C. over reconstitution at 21 to 25° C. could not be explained by an increase in the solubility of the powder, nor an increase in the dispersion of the cells.

"Reconstitution of dried *L. bulgaricus* at 50° C. with subsequent inoculation into skim milk showed greater activity in the skim milk, particularly in the early stages of growth, than was obtained when the culture was reconstituted at 21 to 25° C.

"Freeze-drying part of a skim-milk culture that was also spray-dried showed that cells in the freeze-dried culture were not only activated by reconstitution at 50° but that this temperature actually was lethal to many of the cells. This suggested a physiological difference between freeze-dried and spray-dried cells of *L. bulgaricus,* since the latter were markedly activated by heat." D.P.G.

93. **Spotting and Evaluating Biological Dirt.** M. C. Jamieson, H. J. Forster, and A. Rey. Canad. Dairy and Ice Cream Jour., *26,* 1: 28. Jan., 1947.

Biological dirt ordinarily is invisible dirt that contains bacteria, spoils food products, and sometimes causes sickness and even death. The suitcase type laboratory developed for assisting eating and drinking establishments in sanitation problems (Abs. no. 446, Jour. Dairy Sci., *29,* 12: A206, Dec., 1946) has been extended to the dairy industry. "Jamieson's Sanitation Kit" has been used by a few dairies and by the Manitoba Department of Public Health. The test is simple and requires little technical training or scientific interpretation. The representative area of surface is swabbed off; the swab is smeared over the medium prepared in convenient, small, screw-capped bottles and incubated at room temperature for 3 days. The fewer the colonies that develop, the cleaner the equipment. All plant employees see the results and take an active interest in producing clean culture bottles. Application of this test for farm use is promising. H.P.

94. **Vitamin A and Carotene Content of Ontario Butter.** W. H. Sproule, F. W. Hamilton, C. E. Lackner, S. H. Jackson, T. G. H. Drake, and M. Moffat. Canad. Dairy and Ice Cream Jour., *25,* 12: 23. Dec., 1946.

The mean vitamin A potency of butter from 21 creameries representing the five geographical regions of Ontario was 13,269 I.U. per pound. The monthly mean for the period May to November was 14,702 I.U. and from

December to April 9,915 I.U. per pound. During the periods mentioned, carotene supplied 28.8, 25.2, and 17.6% of the total vitamin A potency of Ontario butter. The vitamin A and carotene contents of butter vary with the month of the year as well as with different regions of production, depending primarily upon pasture supplies. The peak values for both vitamin A and carotene were reached in the month of June, followed by a downward trend during the warm midsummer and early autumn period. The latter value returned to almost the June level in October. A slight decline took place in December, which continued throughout the winter, when the lowest values for the year were recorded. H.P.

CHEESE

95. A Study of Canadian Cheese on United Kingdom Markets. Wm. C. Cameron. Canad. Dairy and Ice Cream Jour., *26,* 1: 33. Jan., 1947.

There is a market in Great Britain for select cheese of uniformly high quality, for which a premium will be paid. To reach this objective, the three main points which warrant special care and attention are: (1) clean milk supply, (2) amount of acid in the finished cheese, and (3) closeness of body. Excellent cheese is one having a close-boring, meaty texture with a clean, nutty, cheddar flavor. Two major defects found in Canadian cheese were openness and curdy body, the latter varying from a dry texture to a chalky or pasty texture. It was generally agreed that in most cases where openness was found the curd had not been sufficiently matured on the pan before milling and later, before salting. It is claimed that too much "added acid" in the form of starter is detrimental to the Cheddar cheese. Not over 1.5% starter is recommended if the cheese is not to become acidy on aging. H.P.

96. Give the Cheese of the Lower Fat Type the Place Due It—20% Steppe Cheese. F. L. (Frank Lambertsen), Nordisk Mejeri-Tidsskrift, *12,* 6: 109–110. 1946.

Cheese made from partly skimmed milk should be produced more than it actually is. This cheese always will have a market because it is cheaper than cheese made from whole milk and it contains the same amount of the nutritionally important solids, protein, and calcium salts.

Steppe-cheese is a square-formed cheese, weighing from 11–13 lbs., with an elastic consistency. Cut, it shows evenly distributed eyes about ¼ in. in diameter. A method that has been used for making this cheese for many years in a Danish factory is given. The milk used is pasteurized. Before the renneting, 2–3% starter, 5% water, and calcium chloride (if necessary) are added to the milk. Rennet at the rate of 230 cc. per 1,000 lbs. of milk

(50 cc. per 100 kgs.) is added at 97° F. and the setting period is 40 min. The curd is cut by 1-cm. knives and stirred for 20 min., after which a little more than half of the whey is drained off. Now the curd is stirred vigorously by a fork and heated quickly to 106° F. by adding boiling water, while the vigorous stirring is continued. Some more whey is drained off and the stirring of the curd finished within about 40 min. Salt, 2.5 lbs. per 1,000 lbs. of milk (250 g. per 100 kgs.), is added to the water used for heating. The stirring finished, the curd is cooled down to 99° F., drawn to the end of the vat, and piled for about 20 min., after which it is cut in blocks, hooped and pressed in a cheese press for 45 min. After pressing the cheese is placed in cold water over night and next morning in brine for 72 hr. After a short period in a cooler at 61° F., the cheese is finished at 54° F. and 80% humidity. The cheese can be stored for 7–8 months. T.K.

CHEMISTRY

97. Colorimetric Determination of DDT in Milk and Fatty Materials.

M. S. Schechter, M. A. Pogorelskin, and H. L. Haller, U. S. Dept. Agr., Agr. Res. Admin., Bur. of Entomology and Plant Quarantine, Beltsville, Md. Analyt. Chem., *19,* 1: 51–53. Jan., 1947.

Recent work has shown that contamination of milk, butter, eggs and meat may result when farm animals consume DDT-treated feed; that ingested DDT accumulates as such in the fatty tissues of experimental animals and can be excreted in milk; and that such milk may become toxic enough to kill other animals drinking it. This report describes a procedure for the determination of DDT as such in foodstuffs containing considerable amounts of fatty matter. The method is not rapid but it permits the detection and determination of DDT in milk in quantities as low as 1 p.p.m. Milk is extracted with Skellysolve B, the emulsions are broken by means of a centrifuge, and DDT is separated from the fatty fraction by a sulfuric acid treatment based on the solubility of fats and the insolubility of DDT in concentrated sulfuric acid. The DDT residue is nitrated and DDT is determined by spectrophotometric measurements. B.H.W.

98. Estimation of DDT in Milk by Determination of Organic Chlorine.

R. H. Carter, U. S. Dept. Agr., Agr. Res. Admin., Bur. of Entomology and Plant Quarantine, Beltsville, Md. Analyt. Chem., *19,* 1: 54. Jan., 1947.

The method presented describes a procedure for estimation of DDT in milk and butter samples by determination of the total organic chlorine. The method is rapid, simple, and reasonably sensitive, but it is not specific for DDT. The sample is extracted with ethyl ether and Skellysolve B, the fat in the extract then is saponified and removed from the mixture, and the aqueous filtrate extracted and subjected to a determination of chloride ion by any standard procedure. The amount of DDT is calculated by multiplying the amount of chlorine by 2. B.H.W.

99. **Recovery of Lactic Acid from Dilute Solutions.** A. A. DIETZ WITH E. F. DEGERING, Purdue Univ., Lafayette, Ind., AND H. H. SCHOPMEYER, Amer. Maize-Products Co., Roby, Ind. Indus. and Engin. Chem., Indus. Ed., *39,* 1: 82–85. Jan., 1947.

Lactic acid of varying degrees of purity may be obtained by solvent extraction, steam distillation and crystallization of its salts. The recovery of lactic acid by passing vapors of an alcohol through a partly concentrated lactic acid solution previously has been reported. A pure grade of acid may be prepared by the hydrolysis of an alkyl lactate. In the present investigation a study was made of the recovery of lactic acid as an ester directly from dilute solutions. The acid is converted to an ester and is extracted with a solvent in which it is preferentially soluble. Certain chlorinated hydrocarbons were found to be selective solvents. The preparation of ethyl and propyl lactates with 1,2-dichloroethane as the solvent is described. The esters can be purified to any desired degree by distillation. B.H.W.

100. **Applied Ultraviolet Spectrophotometry of Fats and Oils.** B. W. BEADLE, Res. Lab., American Meat Institute, Univ. of Chicago, Chicago, Ill. Oil and Soap, *23,* 5: 140. May, 1946.

The application of spectrophotometry to the analysis of fats and oils is described. It is especially useful in studying the changes in the double bond systems of fatty acids. The method is applicable to routine analytical work in connection with the processing of oils as well as to academic studies on the composition of naturally occurring fats and oils. The determinations of small amounts of fatty acids with two or more double bonds or of small amounts of conjugated bonds are possible by this method because of its high sensitivity. J.L.H.

101. **The Oxidation of Methyl Oleate. I. The Preparation, Properties and Reactions of Methyl Hydroperoxido Oleate.** C. E. SWIFT, F. G. DOLLEAR, AND R. T. O'CONNOR. So. Regional Res. Lab., New Orleans, La. Oil and Soap, *23,* 11: 355. Nov., 1946.

The above work was designed to test reports in the literature that methyl hydroperoxido oleate is a product in the oxidation of methyl oleate. Methyl hydroperoxido oleate was separated by low temperature fractional crystallization from partially oxidized methyl oleate. Certain characteristics of the original hydroperoxido and its reaction products are described which "lend definite support to the view that the first oxidation product of methyl oleate is a mixture of 8- and 11-hydroperoxido octadecenoic acid, at least under the conditions employed, *i.e.*, oxidation under the influence of ultraviolet light or reaction at temperatures up to 60° C." J.L.H.

102. Flavor Reversion in Soybean Oil. II. The Effect of Atmospheres of Different Oxygen Concentrations on the Flavor Reversion of Soybean Oil.

Calvin Golumbic, C. J. Martin, and B. F. Doubert, Dept. of Chemistry, Univ. of Pittsburgh, Pittsburgh, Pa. Oil and Soap, *23*, 11: 360. Nov., 1946.

Samples of soybean oil were treated with slow-moving streams of oxygen, tank nitrogen containing 0.5% oxygen, or nitrogen purified by passage over heated copper turnings. The samples were maintained in a water bath at 45.5° C. in Petroff culture flasks. A 250-watt G.E. reflector-drying lamp was placed 3 in. directly over the flask and allowed to act during the flowing of the gas.

It was found that the oxidation rate of soybean oil could be varied over a considerable range without influencing the organoleptic evaluation of the degree of inversion. Even the low rate of oxidation attained by the use of purified nitrogen failed to influence the tendency to revert. When low oxygen concentrations were used, the typical grassy reversion flavor was accompanied by a disagreeable and persistent drying after-taste not readily detectable in the soybean oil reverted in air or oxygen. Reversion under nitrogen ocurred at very low peroxide values. J.L.H.

103. Flavor Reversion in Soybean Oil. III. The Preparation and Flavor Characteristics of a Simulated Soybean Oil.

Calvin Golumbic, A. I. Schepartz, and B. F. Daubert, Univ. of Pittsburgh, Pittsburgh, Pa. Oil and Soap, *23*, 12: 380. Dec., 1946.

Fatty acids were prepared from Neofat, olive oil, cottonseed oil, and linseed oil. The fatty acids were mixed in the proportion in which they occur in soybean oil and were esterified with glycerol. The resulting simulated soybean oil was tested for flavor inversion, a defect common in soybean oil. The flavor produced in the simulated oil by heat and light treatment was distinctly different from the flavor appearing in soybean oil subjected to the same treatment. The results "tend to indicate that the ordinary fatty acid constituents of soybean oil are not entirely responsible for the flavor characteristics of reverted soybean oil. Likewise the hypothesis that linolenic acid is the sole causative agent does not appear likely although it is possible that this acid contributes to the flavor instability of soybean oil." J.L.H.

104. Evaluation of Tests for Rancidity in Edible Packaged Oils.

John E. W. McConnell and W. B. Esselen, Jr. Food Tech. Dept., Mass. State College, Amherst, Mass. Oil and Soap, *23,* 12: 369. Dec., 1946.

In this study oils were stored in sealed containers and later subjected to several tests in order to evaluate the reliability of these tests for quantitative measurements of the extent of rancidity (oxidative). The organoleptic test was found to be the most satisfactory method for determining the quality of corn and cottonseed oils which had been aged in sealed containers.

A high storage temperature or the presence of air influenced the fading time of methylene blue. In sealed containers the aldehyde formation in oils was very slow despite extensive development of rancidity. In oils exposed to air, the aldehydes increased. The results obtained on the changes in film pressure of monomolecular film of corn or cottonseed oil indicate that the change in pressure is not wholly dependent on the organoleptic quality of the oil but is influenced by the oxidation products formed in the presence of excess air.

The oils aged in the dark at 100° C. developed rancidity rapidly, whether stored in sealed containers or exposed to air. Little color change took place in the oils in sealed containers; those exposed to air darkened at first, after which bleaching occurred. Samples exposed to sunlamps at 38° C. deteriorated rapidly in organoleptic quality, whether exposed to air or not. Bleaching occurred in the sealed tubes whereas exposure to air slowed up this action appreciably. This indicates that light is the main factor in fading, while the darkening is caused by the excess oxygen and is accelerated by heat. The implications of these facts in the chain of reactions resulting in rancidity are discussed. The induction period of oils stored in sealed containers was found to be dependent upon the original peroxide value of the oils which, in turn, was influenced by exposure to light. Exposure to light resulted in destruction of peroxides. The chlorophyll value of an oil was found to be governed primarily by exposure to light rather than to its organoleptic state. Its general use is not regarded as a reliable test for rancidity. J.L.H.

105. Cleaning Procedure for Babcock Test Bottles. C. W. Rink. Canad. Dairy and Ice Cream Jour., *26,* 1: 30. Jan., 1947.

Calcium sulfate film in Babcock test bottles can be eliminated with a concentrated solution (30%) of caustic soda. The Babcock bottles are filled with the caustic soda and heated to boiling in a water bath for 30 min. The bottles are left in the bath for another 30 min. after the heat has been turned off. The caustic soda then is emptied and the bottles rinsed with water. If a haze remains, it can be removed with dilute hydrochloric acid or full strength vinegar. The 30% caustic soda can be reused for about three treatments. H.P.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

106. **Milk Powder Production Quality and Marketing.** H. F. George. Canad. Dairy and Ice Cream Jour., *26*, 1: 68. Jan., 1947.

Good powdered milk has the qualities of any other form of milk. The ice cream and dairy industries will use considerably less powder in the near future than they recently were using. Powder used for standardizing will be replaced by fresh skim milk. Some extra solids for ice cream mix and for coffee cream will be needed, plus small amounts for standardizing purposes. The largest users of powdered milk will be the bakers. They will have to be educated to use more powdered milk. The solution to the marketing problem is to sell more powder to the bakers, both through personal contact and practical demonstrations, and to publicize the products for their high nutritional values. H.P.

FEEDS AND FEEDING

107. **Methods of Making Potato Silage and Tests of Its Feeding Value for Dairy Cows.** J. B. Shepherd, T. E. Woodward, and C. G. Melin. U. S. Dept. Agr. Tech. Bul. 914. 14 pp. May, 1946.

Feeding trials with several lots of potato silage, ensiled without preservative, with salt, with ground corn, or with varying amounts of mixed orchard grass and clover hay are reported. Best results were obtained when potatoes were ensiled with hay; such silage was very palatable and cows made good gains on it and maintained milk production. Ensiling potatoes alone or with salt or corn meal did not prove practical. Raw chopped potatoes also were fed with satisfactory results when the allowance was not more than 4 lbs. daily per 100 lbs. liveweight. When the quantity of potatoes is small, they can be fed raw to best advantage. Potatoes can be preserved satisfactorily as silage along with 20 to 25% of good quality hay or may be put into the silo along with corn or other green crops. However, not more than 500 lbs. of potatoes should be used for each ton of green crop; a crop such as corn should be well matured; hay crops should be wilted to not more than 60% moisture. Tower silos should be well reinforced because of the heavy weight of potato silage. Such silage should be fed after milking and along with other roughage of good quality so that the ration will contain sufficient fiber, fat, minerals, and carotene. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

108. **The Digestibility of Fats—A Correlation of Experimental Data.** Karl F. Mattil, Res, Lab., Swift and Co., Chicago, Ill. Oil and Soap, *23*, 11: 344. Nov., 1946.

Reported data in the literature on the digestibility of fats fed to human adults, human infants, and white rats have been subjected to statistical analysis and correlation coefficients calculated. A positive correlation (+0.77, calculated for 16 fats) exists between digestibilities found in human adults and those found for corresponding fats in albino rats.

The correlation coefficient for the relationship between digestibility coefficients and stearic acid content of fats was found to be negative (−0.80 for human adults for 40 fats; −0.77 for human infants for 16 fats; −0.86 for albino rats for 26 fats). The amount of saturated acids of 18 carbon atoms or more that a fat contains is the chief limiting factor of its digestibility.

The coefficients of correlation for the relationship between digestibility and melting point are not as high as those for digestibility and stearic acid content, being −0.66 for human adults for 37 fats and −0.42 for albino rats for 24 fats. This lesser degree of correlation is due to the fact that the melting point is partially a function of the amount of long chain saturated acids.

J.L.H.

109. The Rôle of Various Substances in Stabilizing Animal Tissues.

G. O. BURR, W. O. LUNDBERG, AND J. R. CHIPAULT, Div. of Physiological Chemistry, Univ. of Minn., Minneapolis, Minn. Oil and Soap, *23*, 12: 382. Dec., 1946.

The diet exerts an important influence on the oxygen uptake of body fat. The fats are influenced by their fatty acid composition and the presence of antioxidants or prooxidants. The most clear-cut demonstration is obtained by feeding or withholding tocopherol to rats. Tocopherols were found to differ among themselves in their effect on the keeping time of body fat. The alpha and beta form are twice as effective as the gamma form when fed to rats. The gamma form is several times as effective as an antioxidant as the alpha form when added directly to rendered body fat.

The type of fat in a purified diet was found to be very important. Butterfat was much more effective than lard in increasing the keeping quality of body fat of rats.

J.L.H.

110. The Rôle of Proteins in Animal Nutrition.

H. C. SCHAEFER, Ralston Purina Co., St. Louis, Mo. Oil and Soap, *23*, 12: 375. Dec., 1946.

The rôle of proteins in animal nutrition is discussed. It is emphasized that adult ruminants are not as specific in their protein requirements as the single stomach or monogastric animals. The calf, in early life, is like the non-ruminants in that the rumen is undeveloped; hence it requires a better-quality protein in early life. The nutrition of small-stomached animals is concerned with amino acid nutrition or similar compounds, rather than with protein. More information is required on the nutrients or compounds furnished by proteins of high biological value and better methods are needed for the proper evaluation of proteins.

J.L.H.

ICE CREAM

111. Rôle of Emulsifiers in Ice Cream Making. B. I. Masurovsky. Ice Cream Trade Jour, *42*, 12: 72. Dec., 1946.

Egg yolk, monoglycerides, and diglycerides are being used as emulsifiers for ice cream. To evaluate a commercial preparation as an emulsifying agent, the following test is suggested: Prepare a 15% sugar solution containing 10% butterfat. Introduce 0.25% of stabilizer and 0.25% of emulsifier into the sweetened oil and water system. Apply heat to insure the solubility of the emulsifying agent. Pour the entire mixture into a graduated cylinder, agitate it for 2 min., and allow it to stand undisturbed for 10 min. Examine the volume of free milk fat in the column and calculate the amount of fat emulsified. Compare it with a control mixture without an emulsifying agent.

Ice cream stabilizers sometimes contain emulsifying material, and it is advisable to follow directions given by the manufacturer of these products. The trend seems to be toward the increased use of emulsifiers in ice cream in an attempt to produce a higher quality product. W.H.M.

112. Problems Arising from Increased Costs. Ridgway Kennedy, Jr., Abbotts Dairies, Philadelphia, Pa. Ice Cream Trade Jour., *42*, 10: 118. Oct., 1946.

During the war years the cost of ingredients used in the manufacture of ice cream showed a marked increase. Costs such as delivery, sales, and merchandising have been held in check because of increased volume of sales and government restrictions. Production costs have risen because of increased labor costs and the type of labor available. After price controls were removed, the ice cream manufacturer could raise his price to offset increased costs. During the past year manufacturing overhead costs have been favorable, due to increased volume. However, they will go up if volume begins to go down.

Should the ice cream manufacturer go back to daily delivery and increase the number of flavors carried in stock, or spend excessive amounts of money to get new business, costs are sure to rise. In order that sales volume may be maintained, ice cream manufacturers might well consider the expenditure of money on advertising campaigns designed to increase consumer acceptance for ice cream. Money put into state and national nutrition programs will tend to increase per capita consumption of ice cream and by so doing, operating costs may be reduced. New competition from within and without the ice cream industry will make it necessary for ice cream manufacturers to make decisions based on sound business judgment. W.H.M.

113. Preserved Fruits—Preserve Flavor. F. I. HUTCHINS, Hutchins Advertising Co. Ice Cream Field, *48*, 6 : 30. Dec., 1946.

The manufacture of fruits, nuts and flavors represents a large industry which caters to the ice cream manufacturer. The trend is away from highly colored and extract-flavored products. Processed (*i.e.,* heated) flavors and fruits are considered more sanitary than fresh or cold packed fruits or "dry nuts". W.C.C.

114. Causes of Shrinkage in Ice Cream Making. B. I. MASUROVSKY. Ice Cream Trade Jour., *42*, 9 : 58. Sept., 1946.

Large air cells, heat shock due to sudden change in the temperature of ice cream cabinets, large ice crystals, utilization of certain types of milk solids such as those present in frozen cream, use of certain types of cocoa, improper blending of ingredients, and subjecting the ice cream to a wide range of temperature changes in storage are some of the causes of shrinkage. W.H.M.

115. Factors Affecting Shrinkage. R. J. RAMSEY, Ramsey Laboratories, Cleveland, Ohio. Ice Cream Trade Jour., *42*, 12 : 46. Dec., 1946.

Shrinkage in ice cream may be affected by the following factors: (1) The destabilizing effect of freezing upon the colloidal suspension of proteins surrounding each air cell. (2) Low viscosity in the frozen ice cream, which may be influenced by ice crystal structure, sugar, type of freezer, and condition of the ice cream. (3) Air cell structure (fine air cells actually cause ice cream to shrink more readily than large air cells). (4) The use of dry ice. (5) The use of untreated paper in ice cream cans, boxes, or cartons. (6) Freezer operation. More shrinkage occurs in ice cream that is frozen too stiff and too dry at the freezers. . (7) Air pockets due to poor filling practices. (8) High overruns. (9) Increased air circulation over cans. (10) Composition of ice cream. High butterfat, high sugar, excessive amounts of corn sirup, and the use of eggs seem to increase the tendency for ice cream to shrink. W.H.M.

116. Planning the Modern Ice Cream Plant. JOHN W. FARLEY, Sales Engineering Dept., Cherry-Burrell Corp. Ice Cream Field, *48*, 6 : 18. Dec., 1946.

A consideration of small to medium combination milk and ice cream plants is presented. The general over-all purpose of planning such a plant is "To process the maximum quantity which can be sold of the highest quality products at the lowest possible cost".

The following factors are considered as important in the order listed: (1) amount of raw material to be handled, quantity of finished products to be produced, as well as personnel and time available for required operations; (2) quantity of tools and equipment required, as well as expected methods of processing and handling; (3) location of building; (4) size and type of building to be constructed; (5) type of construction to be employed; (6) basis for deciding room arrangement in building; and (7) smallest number of changes which must be made in arrangement of equipment without disturbing the original plan. The necessity of surveys and reliable estimates of expected expansion are emphasized. By considering plant operation time schedules, equipment best suited for efficient operation can be selected. Flow plans are discussed from the point of view of efficiency in processing, packaging and storage. In plant layout it is highly desirable to have at least three sides of the building accessible. The use of a building accessible from only one side "almost invariably means a rather inefficient layout".

W.C.C.

117. **Bulk-Gallon Sales Spreading.** ANONYMOUS. Ice Cream Trade Jour., *42*, 10: 96, 180. Oct., 1946.

The sale of bulk ice cream in single gallon containers for home use, which was started in March, 1946, by the Breyer Ice Cream Company in the Harrisburg, Pa., area, now has been expanded to other areas. Other ice cream companies now sell bulk ice cream by the gallon for home use. A label is placed on the container showing the retail price and how to store and dip the ice cream. Dealers are taking a 26% markup on this item. W.H.M.

118. **Trends in Ice Cream Advertising and Sales.** E. L. WALKER, Arden Farms, Los Angeles, Calif. Ice Cream Trade Jour., *42*, 12: 60. Dec., 1946.

Selling is an art, not a science. It is simply common sense and sound, fast thinking applied to business problems. The trend is away from teaching salesmen too much theory. Show them successful principles in action. Give them facts. Teach them to deal with specific situations. Train them in all phases of operation, including making mixes, freezing, novelties, and delivery. They should be familiar with restaurants, hotels, food markets, and drive-in markets. Ice cream manufacturers should see that their dealers play fair with the public and strive to give them good value for their money. Better package identification in cabinets also is helpful in getting better consumer acceptance. The trend is toward better balanced ice cream advertising campaigns, employing all media such as radio, newspapers, billboards, deluxe boards, point of purchase, car cards, and direct mail.

New possibilities for profitable ice cream mechandising are opening up every day. Some of them are: frozen food stores, vending machines, dairy departments, ice cream cabinets in apartment houses, complete frozen meals with ice cream, complete cooked meals with ice cream, air lines, home and farm deep freeze unit sales, push carts, retail trucks, bicycles and boxes, modern power scooters, liquor stores for package sales, theater lobbies, and cabinets in drugless drug stores and in regular drug stores other than at fountains. Packaged sales, cups, and bars are becoming more popular; frosty malts also are on the upswing. W.H.M.

119. **Super-Market Merchandising.** VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 12: 34. Dec., 1946.

The anticipated entry of food chains and independent food stores into the ice cream marketing picture obviously is underway in all sections of the country. A striking example of how one well known wholesale ice cream manufacturer has reached out for important super-market ice cream volume is the case of the Bettar Ice Cream Co. of Baltimore, Md. The company supplies cabinets and the necessary promotional and advertising signs to 50 stores. The only item sold is the "Luxury Pint", which retails at 30 cents. The insulated bag for carry-home sales is an essential part of the program. A cardinal point in the sale of ice cream through food chains is that the ice cream be sold and merchandized through a department separate from that which sells frozen foods. W.H.M.

120. **Modern Ice Cream Store Planning.** DON MACK, Weber Showcase and Fixture Co., Los Angeles, Calif. Ice Cream Trade Jour., *42*, 12: 39. Dec., 1946.

The six basic layouts for retail ice cream stores include the straight counter, the horse shoe counter, the island arrangement, the full service, the self-service, and the drive-in. Successful store operators follow these rules: (1) Select a store on the shady side of the street in the afternoon, and on the right side of the street on the way home from the heart of town. (2) Select a location where there are at least 300 families within a radius of 10 blocks. (3) See that the floor is attractive, of a usable surface, and that the ceilings and walls are of a light finish. (4) If you are in the same room with other merchants, insist upon the same side of the room that the staple items are merchandised from. (5) Select fixtures which will provide the greatest convenience to your customers, and provide the best working conditions for your employees both from the standpoint of speed and personal comfort. (6) Buy your supplies from wholesalers and jobbers who have a reputation for handling only the best. (7) Send announcements by mail to the 300 families in your neighborhood 3 or 4 days in advance of the opening of a new store, and at frequent intervals when you are going to offer particular flavors or special dishes with a varied appetite appeal. (8) Keep your store and equipment spotlessly clean and never under any circumstances allow a customer to leave your store dissatisfied, even if you have to take a temporary loss.

These rules do not apply in country towns with regard to location. In the country the best locations are near the busiest stores and on the same side of the street. W.H.M.

121. The Merchandising Power of Sanitation. GEORGE HENNERICH. Ice Cream Trade Jour., *42*, 10: 112. Oct., 1946.

The ice cream manufacturer and his dealers must assume responsibility for the sanitary service of ice cream, as people are more conscious of cleanliness than ever before. The first step should be the development of sanitary routines at the soda fountain and ice cream departments through which ice cream is sold to the public. Sterilization of glassware between each use, clean dispensers, clean towels, use of dipper pads, and clean water in the dipper wells are routines which should be done correctly. The motto of every retailer should be "Be Clean, Keep Clean, Serve Clean". W.H.M.

122. Glorifying the Pint Package. VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 10: 102. Oct., 1946.

The Riviera Ice Cream Co. of California operates more than 30 stores and also sells to dealers. It produces only pint packages and controls the retail price by billing the ice cream to dealers at the full retail price less a percentage discount which represents the dealers' markup. Stores operated in Los Angeles are distinctively designed, handle only ice cream, and are operated by one girl. A high butterfat ice cream containing about 60% overrun is sold for 35 cents per pint. Jiffy insulated bags with dry ice are furnished to customers for a 5 cent deposit, which is refunded if the bag is returned five times for refills. Ten different flavors are sold. W.H.M.

123. Ice Cream on Retail Milk Routes. ANONYMOUS. Milk Dealer, *36*, 4: 42, 66. Jan., 1947.

The Adohr Milk Farms, Los Angeles, is successfully distributing ice cream on its retail milk routes. The ice cream is carried on the delivery routes in refrigerator boxes. These boxes hold 24 pints of ice cream and are refrigerated with 2.5 lbs. of dry ice. The ice cream is packaged only in pints. Vanilla and chocolate are the only flavors regularly packaged.

C.J.B.

124. The Ice Cream Industry and Frozen Foods. VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 10: 100. Oct., 1946.

"The main ingredients of success in frozen food distribution are how well you are prepared to merchandise, sell, and service and remembering, all the time, that frozen foods belong to the food business and yet are different than anything heretofore seen in the food industry." Actually, the frozen food business is six different businesses representing fruit, vegetables, fish, meat, poultry, and food specialties. It is a separate business from ice cream making and to be successful it must be operated as such. Any ice cream manufacturer going into the frozen food business should plan for all-year service and merchandising, but he also should have a separate organization for that purpose. W.H.M.

MILK

125. Some Angles on Leveling Milk Production.

C W. PIERCE, Pittsburgh District Dairy Council, Pittsburgh, Pa. Milk Indus. Found. Assoc. Bul., *39*, 3: 56–68. Jan. 3, 1947.

Increased milk production during the fall months will be needed for several years due to population increases, prospective high demand, and larger business activity associated with good incomes. A large recurring fall shortage is likely to result in enlarging a city milk shed to the point where later conditions might unduly reduce the price of milk to the producer. It is to the interest of the producer to avoid severe shortages of milk. Better feeding and care will help, but the greatest leveling effect comes from a change in the breeding program. Spring freshening continues to predominate because farmers think such milk is produced more cheaply, even though cost records apparently show that fall freshening produces lower cost milk. A survey of several hundred farmers in Pennsylvania showed many believed fall and winter costs are higher than spring and summer costs by $1.09 per 100 lbs. It is concluded that an incentive fall price of less than $1.00 over the spring price would not greatly increase fall milk production. A higher class I seasonal price of the equivalent of 2 cents per quart initially, with perhaps 1 cent after enough production is obtained, is favored by the author over the "base rating" plan. E.F.G.

126. Off-Flavored Milk Due to Production Methods.

C. W. ENGLAND, Highland Dairy Farms, Washington, D. C., and Baltimore, Md. Milk Dealer, *36*, 4: 118–120. Jan., 1947.

The causes of off-flavor in milk are discussed. The discussion is summarized as follows: Off-flavored milk due to production methods can be avoided by eliminating the cause. Avoid feed flavors by bringing the cows off pasture-type feeds (or weeds) 3 to 7 hr. before milking. Strong flavored feeds, fed in the barn, should be fed after milking. Prevent flavors due to bacterial growth by practicing proper methods of sanitation and cooling. Eliminate off-flavors due to chemical composition changes by eliminating the guilty cows from the milking herd. Last, keep foreign materials out of milk.

Don't lose sight of the fact that people aren't going to drink milk unless they like it. C.J.B.

127. A Survey of Milk Bottle Costs, Disappearance and Trippage. J. M. McAirty. Canad. Dairy and Ice Cream Jour., *26*, 1: 54. Jan., 1947.

The rate of new glass purchases varies roughly in proportion to population; it is quite probable that the length of delivery routes also is a factor. Other factors which may throw the average out of line are: (1) faulty equipment in one or more of the larger plants, (2) disregard for 5-cent deposit charge, (3) carelessness in handling bottles, and (4) costly system of exchanging bottles between dairies.

The 5-cent deposit decreased the rate of purchase of new bottles and led to the resurrection of used bottles from cellars and back yards. In 1945 in 13 plants in eight cities, the average number of trips per bottle was 5.8 and the average disappearance rate 1.72%. The universal bottle has reduced breakage and loss and has reduced expenses by elimination of sorting and exchanging bottles. H.P.

128. The Treatment of Cream. Trygve Langslet, Malkeforsyningen, Oslo. Nordisk Mejeri-Tidsskrift, *12*, 7: 129–132. 1946.

I. The sanitary treatment of the cream.

The methylene blue test alone is insufficient for good control of the quality of milk and cream. Milk containing millions of bacteria of the type F3 (related to *Bacterium coli* and *Bacterium fluorescens liquefaciens*) has had a reduction time of more than 5.5 hr. by the methylene blue test. Lactic acid bacteria reduce methylene blue but do not grow on peptone agar; with the majority of the foreign bacteria the opposite is true. A combination of the methylene blue test and plate count on peptone agar gives an excellent evaluation of the bacteriological quality of the milk. A determination of the coli count in the milk received ought to be made at the same time.

Control when the milk is received is not enough. Samples for bacteriological control should be taken of the milk on its way through the whole pasteurization system, and from the moment the cream leaves the separator the control should be continued so the bacteriological quality of milk and cream always is known at any place. Small sampling valves can be placed in the system before and after possible sources of contamination.

A higher pasteurization temperature for the milk from which the cream is taken has been used with good results, as well as fast cooling and low storage temperature.

II. The treatment of the cream in order to get as high a viscosity as possible.

The treatment has been adapted from that of Henning and Dahlberg. The best results are obtained by cooling the cream slowly and to as low a temperature as possible after the pasteurization. The cream is heated to between 75.2 and 91.4° F. Above or below these temperatures almost no effect in the viscosity is obtained. The best effect is obtained between 80.6 and 86° F.

A method similar to this one has been used in practice, a different one for cream with different fat content. After pasteurization the milk is cooled to 37.4° F. (not absolutely necessary but the best result is obtained by this) and is heated again to separating temperature. The cream is treated in special vats. Cream containing 35% butterfat or above is separated at 104–111.2° F., cooled to 37.4–39.2° F. in 1 hr., and remains at this temperature 1 hr. Next it is heated to 82.4° F. in 50–60 min. by warm water in the jacket under continuous stirring. Immediately after reaching this temperature the cream is cooled to 46.4° in ¾–1 hr. The cream is drawn in cans and placed in ice for further cooling.

Cream containing 30% butterfat is separated at 95–100.4° F., cooled to 35.6° F. in 1 hr., and kept at this temperature 0–1 hr. before heating to 82.4° F. in 90–110 min. (slow heating). The difference in the temperature between the warm water in the jacket and the cream in the vat must not be more than 18° F. Cooling to 46.4° F. in 1 hr. follows.

Cream containing 20% butterfat or less is separated at 78.8–86° F., cooled to 35.6° F. in 1 hr., and remains at this temperature for 0–1 hr. before heating to 82.4° F. in 110–130 min. (very slow heating). The difference in the temperature between the warm water and the cream must not be more than 9° F. Especially in the beginning the heating must be very cautious. Likewise the cooling from 82.4 to 35.6° F. must be done slowly in 90–110 min., for instance, first by water from 82.4–68° F. and then by brine. The cream is stored in the vat at 35.6° F. until the next day.

The treatment must be adapted for every plant. Bacteriological control of the cream has to made constantly or the cream can be subject to a reduction in quality.

III. The treatment of cream in regard to making it suitable for whipping.

The most important requirements for good whipping cream are:

a. Through the whipping the biggest possible increase in volume shall take place.
b. The cream shall be of a high stiffness.
c. It shall remain stiff for several hours without falling together.
d. The cream shall have a reasonable whipping time.
e. Little leaking of serum, and preferably none, at least not the first hour after whipping.

In order to get a stable product the separating temperature must not be too low. Pasteurized milk may be separated at 95° F. (depends on the season) and give a stable product, but not below 86° F.

Whipping cream ought not to be diluted with skim or whole milk, but with thinner or fatter cream. The temperature of whipping ought to be 44.6–46.4° F.

IV. The packing of cream for sale.

The best thing to do is to sell the cream in bottles or cartons. This way gives the smallest loss and is the most sanitary. T.K.

129. **How to Sell Dairy Products Again.** GEORGE F. BARBER, Abbotts Dairies, Philadelphia, Pa. Milk Indus. Found. Assoc. Bul., *39*, 5: 105–119. Jan. 20, 1947.

A discussion of the selection of routemen is followed by a detailed procedure for conducting a 4-day training school for routemen, using the Milk Industry Foundation training manual, "The Balanced Job", as a guide. The time is divided into not over 15% in lecture, at least 40% in training the men, and 45% in exchanging experiences and opinions of specific problems and cases. The various duties of a routeman are analyzed and definite methods of instruction and procedure are specified. It is recommended that routemen be relieved of route duties for the period of the course, as it cannot be given effectively after a day of regular route service. E.F.G.

130. **What Kind of Plans and Materials are Put to Most Effective Use by Home Service Route Salesmen?** EDWIN FUNK, Sheffield Farms Co., Inc., New York, N. Y. Milk Indus. Found. Assoc. Bul., *39*, 5: 99–104. Jan. 20, 1947.

What helps are furnished to the routeman will depend upon whether a "moderate base, high commission" or "high base, moderate commission" payment system is in effect. A simple natural approach to the problem is advised to get him to use these helps most effectively. A check list of 23 details in producing routemen's sales tools is given under three headings, *viz.*, general, bottle collars and hangers, and folders and circulars. E.F.G.

PHYSIOLOGY

131. **Preparation and Chemistry of Anterior Pituitary Hormones.** ABRAHAM WHITE, Dept. of Physiol. Chem., Yale University, New Haven, Conn. Physiol. Rev., *26*: 574–608. 1946.

On the basis of physiological evidence, apparently at least six recognized individual hormones exist, although there is some biological overlapping among certain of the anterior pituitary secretions. The four most highly purified anterior pituitary proteins are the lactogenic, the adrenotrophic, the growth, and the luteinizing hormones. The thyrotrophic principle has been isolated in highly purified form but has not yet been examined by rigid criteria of protein purity. The follicle-stimulating hormone awaits further purification. D.E.

132. **Mechanism of the Development of Obesity in Animals With Hypothalamic Lesions.** JOHN R. BROBECK, Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn. Amer. Jour. Physiol., *26*, 4: 541–559. Oct., 1946.

Experimental study has shown that the obesity of animals with hypothalamic lesions arises primarily from a marked increase in food consumption, sometimes accompanied by a decrease in locomotor activity and by a transitory depression of basal heat production. The extra food eaten by the animal constitutes a relatively large energy surplus, which the tissues dispose of by storing some of it and by oxidizing the rest to carbon dioxide and water. Since lesions of the hypothalamus induce these highly typical deficits, the hypothalamus probably normally participates in the maintenance of the over-all energy equilibrium; the control of food intake, work output and body temperature may be correlated and integrated within this portion of the diencephalon. D.E.

MISCELLANEOUS

133. **Possible Trends of Dairy Research in Canada.** J. A. PEARCE. Canad. Dairy and Ice Cream Jour., *26*, 1: 64. Jan., 1947.

Possible trends in post-war dairy research in Canada are: (1) the utilization of waste dairy products or dairy by-products as specialty foods, (2) the possibility of using dehydrated whey in baking, and (3) the use of continuously operated dairy equipment, such as continuous butter-making machines and the adaptation of this churn to Canadian composition requirements for butter. Keeping quality studies will have to be made on this butter and results compared with butter made from the conventional methods of manufacture. H.P.

134. **Insect and Rodent Control in Dairy Plants.** GEORGE C. DECKER, Entomologist, Ill. Natural History Survey and Ill. Agr. Expt. Sta. Milk Dealer, *36*, 4: 124–128. Jan., 1947.

In the control of insects and rodents, both preventive and remedial measures should receive thorough consideration. Plant location and construction are emphasized among the preventive measures. The discussion of insect control is based mainly on the use of DDT. It is pointed out that, because DDT is used under greatly varying conditions to control many kinds of insects, it is marketed in several forms. Each has its distinct uses, advantages, and disadvantages. Following is a list of the forms now readily available:

(1) Prepared DDT dusts, ready for use, are available in concentrations of from less than 1% to 10 or 15%.

(2) DDT dust concentrates, containing 25 to 50% of DDT, to be used by jobbers or growers for preparing dilute dusts.

(3) Wettable powders, which are similar to dust concentrates but contain a wetting agent, are intended for use in the preparation of sprays.

(4) Oil solutions of several kinds are available. Some for use as household fly sprays contain as little as 0.2 to 1% of DDT in refined kerosene. Others intended for household use on bedbugs, flies, roaches, etc., contain 5% of DDT in refined kerosene or other suitable solvent.

(5) Emulsion concentrates are solutions of DDT, an emulsifying agent, and a solvent. They can be mixed with water to make sprays.

(6) DDT bombs, or "aerosol" bombs, contain DDT dissolved in liquefied gas. These bombs are for use in homes and other enclosed places.

DDT is used either as space sprays or as residual sprays. The space sprays also contain some quick-acting agent and are highly effective. They are used extensively in enclosed places. With the residual sprays, the residue left on surfaces sprayed or painted with DDT suspensions, emulsions, or oil solutions containing 1 to 5% of DDT will continue to kill flies, mosquitoes, roaches, etc., for from 1–2 weeks to several months after the spray is applied. The use of a 5% DDT solution or spray to be applied at the rate of 1 gallon per 1,000 sq. ft. of surface is generally recommended. DDT is effective in some paints but decomposes rapidly in whitewash.

Rodent control is discussed from the standpoint of prevention as well as poisoning. In addition to the poisons usually used, the uses of Antu (alphanaphthylthiourea) and 1080 (sodium fluoroacetate) are discussed.

C.J.B.

135. **Berlin Diary (Dairy Edition).** HENRY I. TRAGLE. Milk Dealer, *36*, 4: 47–48, 100–106. Jan., 1947.

A description is given of the C. A. Bolle plant in Berlin, which in normal times handled from 150,000 to 250,000 liters of milk per day. The plant had manufactured butter, margarine, ice cream, skim and whole milk cheeses, and a variety of fermented milk products. In addition they had operated a recovery plant where various chemical by-products of milk, such as casein and milk sugar, were extracted from the milk waste. High-temperature, short-time pasteurization was used. A description is given of the plant layout and equipment.

C.J.B.

136. **Future Price Supports for Dairy Products.** DON S. ANDERSON, Dairy Branch, Production and Marketing Admin. Milk Indus. Found. Assoc. Bul., *39*, 3: 49–55. Jan. 3, 1947.

The Act of 1941 and the Steagall Amendment require the support of the prices of certain agricultural products at 90% of parity for 2 years after

Jan. 1 of the year in which the war is officially declared "over". (Abstractor's note—on Dec. 31, 1946, President Truman officially declared the war "over", so this act now supports prices through Dec. 31, 1948.) Little direction was given the Department of Agriculture with regard to how the support was to be effected.

Three possible methods of price support are suggested: (1) Do little about production but dispose of surpluses as can be done best. (2) Put into effect a strong production and marketing program, using supports to encourage shifts in production in predetermined directions. This probably would mean higher support prices for some products and quotas for others. (3) Employ a minimum of controls and some incentives to prevent unmangeable surpluses. The author suggests that any adjustment in the dairy industry probably will be toward more milk production rather than less. E.F.G.

137. **How Efficient Is Your Creamery?** L. C. THOMSEN, Univ. of Wis. Natl. Butter and Cheese Jour., *37*, 12: 38. Dec., 1946.

Changing conditions in the creamery industry require careful evaluation of plant efficiency. A chart is given which may be used for systematic study of factory operations. Factors included are quality, manufacturing methods, personnel, operating losses, accounting, sales, purchases of equipment and supplies, and public relations. Each factor is evaluated by the answers to specific questions which call attention to the important phases of the factor under consideration. W.V.P.

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

138. Ice Cream Industry. Second Edition. G. D. TURNBOW, P. H. TRACY, AND L. A. RAFFETO. 654 pages. $6.00. John Wiley & Sons, New York. 1947.

· This textbook on the manufacture of ice cream is entirely new and replaces an earlier book by Turnbow and Raffeto. The text covers the ice cream industry from its early history up to and including the war years. The book is well illustrated, contains 25 chapters of worthwhile information, and is understandable to the plant man who has had no technical training.

There are chapters dealing with history, classification, and recipes. The recipes (15 pages) are largely for various flavors used and for ice cream of different flavors, sherbets, ices, and fancy ice creams. Adequate directions also are supplied. Other chapters cover composition of mix, selection of milk products, and sweetening agents with their relative sweetness values.

A chapter of considerable size deals with stabilizers in which gelatins, gums of the kinds used in ice cream, and emulsifying agents are discussed. A short chapter is devoted to eggs in ice cream, and another to standardization of the mix. "Mix Preparation" is the title of a long chapter covering equipment use, standardizing of acidity, pasteurization, homogenizing, cooling, restandardization of off batches, making mixes in the vacuum pan, and coloring. Two chapters are devoted to freezing the mix by batch and both kinds of continuous freezers, overrun control, and factors affecting overrun. Flavoring ice cream is covered in Chapter 12, while other chapters deal with hardening ice cream and with packaging and delivery. Ice cream novelties, sherbets and ices, specialties, and the manufacture of fancy ice cream forms are well handled. Several excellent illustrations show how fancy forms are made.

Sanitary control, bacterial content, washing and sterilizing, food value, defects, and physical and chemical properties are discussed in detail in chapters devoted to these subjects. The business phases of ice cream manufacturing are covered in chapters devoted to merchandizing, plant costs, and records. The final chapters deal with mechanic phases such as refrigeration, steam and equipment, the testing of dairy products used in ice cream, and the testing of ice cream for fat and total solids. C.D.D.

139. Prices of Dairy Products and Other Livestock Products. FRANK A. PEARSON AND EDMUND E. VIAL. 154 pages. $3.00. Cornell University Press, Ithaca, New York. 1946.

This book contains the results of a study to determine the effect of monthly production, stocks, supply, price level, demand, and competitive products on the monthly prices from 1920 to 1941 for each of twelve livestock products: butter, cheese, evaporated milk, nonfat dry milk solids, casein, eggs, poultry, lamb, veal, beef, pork, and lard. Oleomargarine and cottonsed oil also are discussed because of their close relationship to butter and lard, respectively. Most of the variations in prices of livestock products are explained on the basis of the general price level and the production, stocks, or supply of the different products. These factors accounted for 45 to 94% of the variation in the monthly prices. The importance of price level varied from month to month. For some products large production and low prices were associated, while in others high prices and high production were related. Stocks generally were related inversely to prices, but the effects varied widely from season to season and from product to product. Butter prices, for example, were more closely related to supply than to production. The commonly used measures of demand—national income, earnings of factory workers, and business activity—did not explain much of the variation in the prices after the effects of the price level and production, stocks or supply had been eliminated.

The book is well written, with most of the data presented in simple tabular and graphical form. A 14-page abstract and summary contains conclusions based on the principal findings for each product and a statement of the economic principles involved. This book should be a welcome addition to the library of any person interested in the factors affecting the prices of dairy and closely related farm products. W.L.S.

140. **Bacterial Chemistry and Physiology.** John R. Porter. pp. 1073 + x. $12.00. John Wiley & Sons, Inc., New York, N. Y. 1946.

This book is a needed addition to the literature of fundamental bacteriology, as it summarizes material from very diverse sources and provides an excellent bibliography for further study of special points. Much of the material is such as to have considerable technological application. Of the ten chapters, those on growth and death of bacteria, effects of physical agents on bacteria, effects of chemical agents on bacteria, bacterial enzymes and bacterial respiration, bacterial nutrition, and microbial fermentations should be of special interest to people in the dairy industry. F.E.N.

141. **Concise Chemical and Technical Dictionary.** H. Bennett, Editor. pp. 1055 + xxxix. $10.00. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1947.

More than 50,000 items, compounds, or names are described or defined. For many chemical compounds the chemical name, synonymous names, semistructural formula, molecular weight, color, form, specific gravity, melting point, and solubilities are given. The biological terms in general are incompletely listed, and many of those listed are not defined as well as one might wish. The coverage of trade names and common trade terms appears to be unusually good and is one of the most desirable features of the book. The rules of nomenclature of organic chemistry adopted by the Council of the International Union of Chemistry in 1930 are summarized in some detail, and an extensive list of names and formulae of radicals occurring in organic compounds is presented. The 1934 report of the Nomenclature, Spelling and Pronunciation Committee of the American Chemical Society is reproduced by permission. Tables of Greek, mathematical, apothecary and miscellaneous symbols are given, as are weights and measures and temperature conversion scales. The list of indicators unfortunately lists those for pH, oxidation-reduction potential, and specific compounds all in one group. The diagraming of many of the important organic ring systems provides a useful summary.

While this is not a handbook in the usual sense of the word, it fulfills many of the functions of a handbook and provides considerable information, especially in the fields of chemistry and related topics, which is not included in the usual handbook. F.E.N.

BACTERIOLOGY

142. A Review of Micrococcus Enterotoxin Food Poisoning. W. C. Haynes and G. J. Hucker, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Food Res., *11*, 4: 281. July–Aug., 1946.

A rather complete summary of the available information relative to the rôle of certain varieties of micrococci in causing outbreaks of food poisoning or gastroenteritis in humans is presented. Milk and other dairy products frequently have been involved as sources of the organisms. Much of the presentation deals with the characteristics and properties of the bacterial enterotoxin, which is the real cause of the symptoms found in those suffering attacks. An extensive bibliography is presented. F.J.D.

CHEESE

143. Building of Store Rooms for Cheese. Frank Lambertsen. Nordisk Mejeri-Tidsskrift, *12*, 11: 221–230. 1946.

The modern store room for cheese must have a capacity large enough that it can hold 3–4 months' production; thus the cheese does not need to be sold in a period of low prices. A one-story building is the ideal, because cheese can be trucked easily from room to room. A one-story store room may be built in the basement and extended out under the area around the factory. The racks should be low enough that a man easily can reach the cheese placed on the upper shelf. A sufficient space between the shelves and between the shelves, ceiling, and floor is of great importance for good working conditions, as are wide passages between the racks. No rack should ever touch the wall. The walls, ceilings, and floors should be smooth and without irregularities. For sanitary reasons, the walls should be faced with brick.

If a one-story store room cannot be built, the staircases and elevators should be placed conveniently. Likewise, the different rooms—the salting room, curing room, the cold cooler, and the sales room—should be located conveniently.

Much is dependent on the temperature and the humidity in the store room. Good insulation and a good heating and cooling system are of great importance. Heating is best done by circulation of warm water. Radiators are best placed on the walls. The cooling system can work with an evaporation temperature of 32° F. or a little above, and the humidity easily can be held at 80–85° F. without artificial humidification. A cooling system in which the air is circulated by a fan is best. It prevents water condensate on walls and ceilings and renews the air.

In store rooms for Blue cheese and Emmenthaler cheese the fan system cannot be used because of the high humidity wanted. Pipes in which a cooling medium is circulated can be placed under the ceiling and cold air will circulate over the cheese and keep it cold.

Both building and equipment must be of good quality. T.K.

144. Process for Making Material for Use in the Manufacture of Process Cheese. Higbee Wayne Bryant (to Kraft Cheese Co.). U.S. 2,392,362. Jan. 8, 1947. (5 claims).

In the manufacture of the class of cheese including Limburger, brick and Camembert varieties, after the curd is matted, it is subdivided into chunks not materially more than 1 inch in thickness and then washed promptly to prevent re-matting. The surfaces are subjected to action of appropriate aerobic ripening organisms in an atmosphere suitable for proper development and the ripened chunks then consolidated by heat to form the finished cheese. Increased rate of ripening under conditions which "substantially prevent" formation of undesirable rind is claimed. F.E.N.

145. Process for Making Cheese. Alan E. Flowers and Andrew E. Merget (to The De Laval Separator Co.). U.S. 2,415,239, Feb. 4, 1947. (4 claims).

A process for removing gas from cheese curd dispersed in whey and centrifuging out the curd as the heavier component by a continuous process is described. F.E.N.

CHEMISTRY

146. The Component Fatty Acids of Buffalo Colostrum Fat. C. P. Anantakrishnan, V. R. Bhale Rao, T. M. Paul, and M. C. Rangaswamy, Imperial Dairy Research Institute, Bangalore, India. Jour. Biol. Chem., *166:* 31–33. 1946.

Composite mixtures of colostrum fat from four Murrah buffaloes taken the first five days, the tenth and fifteenth days of lactation were analyzed for the usual physical and chemical fat constants. The refractive index and the iodine value gradually decreased, with a corresponding increase in the Reichert and saponification values. Fat from the first, second, third, and tenth days of lactation was subjected to detailed chemical analysis by ester fractionation. "The chief changes to be found were the gradual increase in the amount of butyric, myristic, and palmitic acids and a decrease in the amount of stearic and oleic acids, the decrease in the latter being more pronounced." The analytical data are summarized in two tables. A.O.C.

147. The Immune Proteins of Bovine Colostrum and Plasma. Emil L. Smith, E. R. Squibb and Sons, New Brunswick, N. J. Jour. Biol. Chem., *164:* 345–358. 1946.

Colostrum and the protein fractions derived from it were studied electrophoretically. A lactoglobulin possessing all the immune properties of colostrum could be isolated, and data are given to show that this globulin easily is distinguished from β-lactoglobulin. In two different trials this immune globulin comprised 55% of the total protein of colostrum drawn 1 hour post partum. "Immune activity has not been found in fractions free from this protein, and conversely the isolated protein accounts completely for the immune properties of colostrum . . . by the second day the composition of the colostrum begins to approach that of milk and the immune lactoglobulin fraction can no longer be obtained free of other proteins by the simple method described."

Some discussion is devoted to the relationship of the various immune proteins. While the colostrum globulin is similar to the globulin found in bovine blood serum, the two are not identical. A.O.C.

148. Isolation and Properties of Immune Lactoglobulins from Bovine Whey. Emil L. Smith, E. R. Squibb and Sons, New Brunswick, N. J. Jour. Biol. Chem., *165:* 665–676. 1946.

"The high levels of immunity generally present in colostrum have served to obscure the fact that immunity is also present in the later milk. Though present in small amount, the immune proteins occur regularly in the whey of normal animals.

"Electrophoretic analysis has shown that the immune lactoglobulins constitute about 10 per cent of the protein in normal bovine whey. During hyperimmunization the immune components may increase considerably, although this does not occur regularly.

"A method has been described for the isolation from whey of the euglobulin and pseudoglobulin in electrophoretically homogeneous form. Immune activity is associated with both of these proteins.

"The isolated proteins have been studied in the Tiselius apparatus at different pH values, and the proteins have been characterized by their isoelectric points, diffusion constants, absorption spectra, and other properties.

"Studies in the ultracentrifuge reveal that all of the isolated bovine immune proteins contain more than one component. The principal component (76 to 92%) has a molecular weight of about 180,000." A.O.C.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

149. Plastic Cream, Its Production and Uses. R. J. SPIERS, Abbotts Dairies, Inc., Philadelphia, Pa. Dairy Ind. Found. Assoc. Bul., *39*, 7: 189–192. Feb. 14, 1947.

The present methods of producing plastic cream were perfected and patented by Abbotts Dairies, Inc., and a suitable bowl assembly designed by the De Laval Separator Co. A number of factors must be controlled carefully if the quality of the product is to be right. Included are: (1) good quality raw material, (2) freedom from copper or iron contamination, (3) temperature control (170° F. for 15 min. after the first separation and the second separation carried out at 145° F.), (4) product cooled enough to be like ice cream as it is packaged—never so much that paddles are necessary for handling, (5) use of a cylindrical cardboard container similar to 5-gallon ice cream can, (6) cooling as soon as packaging begins, using 10–20° F. air blast, (7) storing at 0 to −10° F., (8) shipping in brine-refrigerated cars, (9) keeping the fat content between 79.5–80.5% (all testing must be done at time of packing and not on frozen cream).

A thorough study of the psychrophylic type of bacteria in this product is needed. Plastic cream may be used for any purposes for which 50% cream is used and for some additional purposes. Largest use is in ice cream, with cream cheese a close second. If more attention is not paid to quality, plastic cream will lose much of the trade advantage it recently has earned.

E.F.G.

150. Manufacture of Casein by Means of Gyratory Motion Applied to an Inclined Screen. EDWARD J. WENDT (to Hercules Powder Co.). U.S. 2,415,268, Feb. 4, 1947. (4 claims).

This patent describes a device consisting of a means for causing gyratory motion to which is attached a closed pan provided with an inlet, a fine-mesh screen on the bottom but tilted from the horizontal, and an outlet in the pan from the highest point on the screen. A catch basin is placed under the pan to collect the liquid after the very fine particles have been separated from it. F.E.N.

151. **Dairy Process.** Gerald C. North and Alvin J. Alton (to Beatrice Creamery Co.). U.S. 2,392,401, Jan. 8, 1947. (6 claims).

Powdered whole milk is made by first separating the whole milk to fluid cream and skim milk. The resultant skim milk is condensed and heated to 170–180° F. for 15–30 min. The cream is heated separately to 170–180° F. for 15–30 min., then combined with the treated skim milk portion and the resultant mixture dried to a powder. F.E.N.

FEEDS AND FEEDING

152. **Factors Affecting the Enzymic Destruction of Carotene in Alfalfa.** H. L. Mitchell and S. M. Hauge, Purdue University Agr. Expt. Sta., Lafayette, Ind. Jour. Biol. Chem., *164:* 543–549. 1946.

"Enzymic destruction of carotene in alfalfa leaves was retarded as long as the tissues remained turgid, but increased rapidly with wilting. Since the loss of carotene was very rapid when the cells had been ruptured or otherwise modified by freezing, it appears that cell permeability limits carotene destruction. Under field conditions, little loss of carotene occurs until wilting takes place.

"Soil fertility had no significant effect on the carotene-destroying activity of alfalfa leaves.

"As the plants approached maturity, there was a slight decrease in carotene-destroying activity.

"There were no consistent differences in the carotene-destroying activity of the four varieties of alfalfa studied.

"Enzymic destruction of carotene during field curing appeared to be greater than destruction by light." A.O.C.

ICE CREAM

153. **Retension of Ascorbic Acid in Strawberries during Processing, Frozen Storage and Manufacture of Velva Fruit.** H. J. Loeffler, Western Regional Research Laboratory, Albany, Calif. Food Res., *11,* 1: 69. Jan.–Feb., 1946.

Prime, mature strawberries averaged 66 mg. of ascorbic acid per 100 g. on a sirup-free basis 3 months after freezing. Immature fruit averaged 91 mg. and over-ripe fruit 55 mg. on the same basis. Whole or sliced berries frozen without sugar lost no ascorbic acid during freezing. With sugar the losses amounted to about 10–15%. Whole berries packed in water lost about 20%, while pureeing or flash pasteurizing of the puree prior to freezing reduced the ascorbic acid not more than 5%.

On short storage (2–3 months), the berries frozen without sugar lost about 12–15% of their ascorbic acid; those with sugar, less than 5%; purees with sugar, 12%; and purees without sugar, about 16%. On extended storage up to 15 months, the sugared and unsugared berries lost only an additional 5% of ascorbic acid. The sugared and unsugared purees lost about 10 and 15%, respectively.

Losses during defrosting were found to be greater than in freezing and holding, particularly if the defrosting was slow or if the products were held some time after defrosting. When sugared puree was made into velva fruit, the loss of ascorbic acid was less than 5%. Unsugared purees, however, lost 12% during the mixing and refreezing. F.J.D.

154. Retension of Ascorbic Acid in Raspberries during Freezing, Frozen Storage, Pureeing and Manufacture into Velva Fruit. H. J. LOEFFLER, Glacier Packing Co., Sanger, Calif. Food Res., *11*, 6: 509. Nov.–Dec., 1946.

Essentially all the ascorbic acid was retained in raspberries during freezing and storage up to 4 months, and not over 25% was lost up to 28 months. Sugar was found of definite value in reducing even this small loss. In frozen raspberry purees, lack of sugar more than doubled the loss of ascorbic acid during 16 months of storage. F.J.D.

155. Ice-Cream Freezer. LEROY H. KNIBB. U.S. 2,416,326, Feb. 25, 1947. (15 claims).

The motor-driven freezer is intended for insertion into the ice compartment of a refrigerator. Provisions are made for discharge to the atmosphere of the air heated by the driving motor. F.E.N.

MILK

156. When Electricity Is Used for Pasteurization Does It Fit in with Other Plant Operations. ISRAEL ADAMS, St. Lawrence Dairy, Reading, Pa. Dairy Ind. Found. Assoc. Bul., *39*, 7: 177–179. Feb. 14, 1947.

The installation of an electrical pasteurizer presents no problems that cannot be corrected as readily as when installing any other system of pasteurization. The possibility of two or three small units to get the desired total capacity is suggested. E.F.G.

157. Pros and Cons of Short-Time High-Temperature Pasteurization.

R. J. WINNING, Sheffield Farms Co., Inc., New York, N. Y. Dairy Ind. Found. Assoc. Bul., *39*, 7: 180–188. Feb. 14, 1947.

The advantages include: (1) a double safety factor from automatic control and the flow diversion valves, (2) lower equipment cost for large plants, (3) less floor space, (4) regeneration savings, (5) less labor in cleaning, (6) easier to operate, (7) more uniform product, (8) elimination of trouble from thermophilic bacteria, (9) expansion at small cost, (10) less milk held too long in case of shut down, (11) lower milk losses, (12) fact that thermoduric bacteria may survive encourages cleaning up milk supply, and (13) equipment more easily sterilized.

Disadvantages are: (1) gasket trouble, (2) some products as buttermilk, sour cream, etc., do not process well, (3) possibility of freezing when a low temperature cooling medium is used, (4) flow diversion valve should be improved and body made of stainless steel.

Some suggestions for use include checking flow in both forward and diverted position, keeping the unit completely airtight for ease of cleaning, and checking the flow with both homogenized and regular milk when putting out homogenized milk. E.F.G.

158. Experiences Using the S.T.H.T. on Milk, Cream, Buttermilk, Cocoa and Cottage Cheese.

MARTIN KLOSSER, Bowman Dairy Co., Chicago, Ill. Milk Ind. Found. Assoc. Bul., *39*, 7: 167–179. Feb. 14, 1947.

Results from STHT as compared with a long flow method are as good from the standpoint of thermodurics, thermophilics, phosphatase test and cream volume, and better for the flavor of the milk. Seven units are operated in four plants of the writer's organization, with a total capacity of 105,000 lbs. of milk per hour. Both the Chicago Board of Health and the plant engineer frequently check the operation of the units in various ways. Sixteen hours with 75% regeneration seems to be the maximum length of run before loss in heat transfer rate requires disassembling and cleaning up the unit. Milk is heated to 161.5° F. for 16 sec. with clarification between regenerator and heater. Homogenized milk is heated to 170° F. for 16–18 sec., going from regenerator to homogenizer to clarifier to heater at 70° F. Cream is cooled in the cooling section. Thirty-six per cent cream takes about 20 sec. rather than 16 to pass through the holder. Skim milk for culture is heated with a second regenerator and heater to 185–190° F. When processing chocolate drink made from cocoa, a meandering retarder gives a 10 min. holding period at 180° F. Cocoa has a grinding effect on pumps, which need to be checked frequently. For cottage cheese 161.5° F. for 16 sec. is high enough; higher temperatures give curds which are too

fine. For cleaning, the alternate acid and alkali methods are used, followed by assembly and a sterilizing water rinse. STHT is used for all fluid milk, specialty products, and by-products, except sour cream and ice cream mix.
E.F.G.

159. **Some Cooking Qualities of Homogenized Milk. II. White Sauces.** Alice M. Towson and G. M. Trout, Michigan State College, East Lansing, Mich. Food Res., *11,* 3: 261. May–June, 1946.

White sauces made with homogenized milk failed to incorporate added fats as well as when made with unhomogenized milk. As the amount of fat was increased, the difference became more pronounced. The viscosity of the sauces increased as the pressure used in homogenizing the milk was raised. Beaten sauces made with a rotary beater were smoother in texture and superior in flavor when homogenized milk rather than unhomogenized milk was incorporated into them. F.J.D.

160. **Improving Milk Quality from Cow to Plant.** C. B. A. Bryant, Johnson and Johnson, Chicago, Ill. Milk Dealer, *36,* 4: 49–54. Jan., 1947.

See Abs. 72, Jour. Dairy Sci., *30,* 3: A33. Mar., 1947.

161. **Bottle Capping Head.** Carl W. Goodwin (to American Seal-Kap Corp.). U.S. 2,416,001, Feb. 18, 1947. (4 claims).

A capping head for use with a hood cap having a central diaphragm, a top wall, and a fluid marginal skirt to be folded around the outer surface of the bottle neck is described. F.E.N.

162. **Method and Apparatus for Pasteurizing Liquids.** Raymond E. Olson (to Taylor Instrument Co.). U.S. 2,415,304, Feb. 4, 1947. (10 claims).

The basic change involved in the usual high temperature–short time pasteurization process is the provision for raising the temperature of the heating fluid to a value above the predetermined level in response to diversion of the product when it fails to maintain the prescribed temperature.
F.E.N.

MISCELLANEOUS

163. **Federal and State Standards for the Composition of Milk Products.** Anonymous. U. S. Dept. Agr., Bureau of Dairy Industry, Leaflet BDIM—Inf—45. 4 pp. Feb., 1947.

Federal, State and Territorial standards in force Jan. 1, 1947, are presented in tabular form, with explanatory footnotes in many instances. Data on minor products are not included. F.E.N.

164. Manufacture of Cream Products. LLOYD K. RIGGS (to Kraft Foods Co.). U.S. 2,414,837, Jan. 28, 1947. (6 claims).

A material containing 80 to 95% milk fat is produced by adjusting cream to pH 3.8 to 4.8, heating to at least about 180° F., and then centrifuging the heated acid cream to break the original emulsion. F.E.N.

165. Sediment Testing Device. BERNARD L. KINYON. U.S. 2,414,044, Jan. 7, 1947. (11 claims).

A portable sendiment tester using vacuum and compressed air for actuating the reciprocal plunger within the barrel is described. A measured quantity of milk is drawn into the barrel on the suction stroke and discharged through the filter on the pressure stroke. F.E.N.

166. Method of Removing and Concentrating Residue from Containers. E. ROY ALLING AND HENNING A. TREBLER (to Rice & Adams Corp.). U.S. 2,418,063, Mar. 25, 1947. (11 claims).

A pre-rinse for a continuous can washer, in which two lots of detergent-free water are used consecutively and repeatedly until a "marketable concentration" of material such as milk is built up in the first of the two rinses. is described. The rinsed cans then are washed with water containing a detergent. F.E.N.

167. Can Dumping Mechanism. CLAUDE H. ABBOTT. U.S. 2,413,900. Jan. 7, 1947. (5 claims).

A power-driven can dumping device for use with milk cans is described. F.E.N.

168. Defrosting Frozen Foods by High Frequency Heat. W. H. CATHCART AND J. J. PARKER, National Bakery Division, The A & P Tea Co., New York, N. Y. Food Res., *11*, 4: 341. July–Aug., 1946.

Utilizing a 3-kilowatt high frequency unit made by the Federal Telephone and Radio Corporation, the authors were able to defrost frozen eggs, fruit, vegetables, and fish in cardboard containers in from 2 to 15 min., depending on the size of the package. This was accomplished without loss of quality, such as discoloration and flavor deterioration. F.J.D.

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ABSTRACTS OF LITERATURE

BUTTER

169. Is Mold Mycelia Count a Satisfactory Index of Butter Quality? P. R. ELLIKER, Purdue University, Lafayette, Ind. Natl. Butter and Cheese Jour., *38*, 3: 38. March, 1947.

The greatest faults of application of the mold mycelia count to butter have been: (1) Its value is only seasonal. (2) Even in the warm months it operates successfully only in certain parts of the country. (3) In Indiana and surrounding regions it does not eliminate the quality inferiority represented by more than 50% of all cooking grade butter and declares about one-third of the 90 score butter illegal during the warm months. Better approaches to quality control might be reached by careful organoleptic grading and sorting and by using mold mycelia counts on finished butter only as a supporting test to grading and testing for extraneous matter. W.V.P.

170. What We Can Do to Increase Butter Consumption. H. SCHUMACHER, Swift & Co., Chicago, Ill. Natl. Butter and Cheese Jour., *38*, 4: 40. April, 1947.

A five-point program is given: (1) Quality is the only safe basis for increasing sales and building repeat business. (2) The product must be properly protected, preferably by a wrapper or coverings more effective than a single sheet of parchment. (3) The package must have eye appeal. (4) Adequate advertising is needed. (5) Displays of butter in modern cases will increase sales. W.V.P.

CHEESE

171. Standards of Identity for Cheese. A. B. EREKSON, Plymouth, Wis. Natl. Butter and Cheese Jour., *38*, 2: 42. Feb., 1947.

Proper standards should protect health, values, quality, and investments of established manufacturers. Pasteurization of milk, suitable aging of raw-milk cheese, and elimination of preservatives are health protection measures. Labeling of skim milk cheese is not adequate protection of values. Specifications should provide maximum moisture and minimum fat contents for each recognized variety or class of cheese. W.V.P.

172. Manufacture of Granulated Type American Cheddar Cheese. E. C. DAMROW, Damrow Bros. Co., Fond du Lac, Wis. Natl. Butter and Cheese Jour., *38*, 4: 44. April, 1947.

Granular type Cheddar now can be made with mechanical equipment and a minimum of hand labor. The regular milled curd process is followed until the time of draining, although a slightly lower cooking temperature may be used to increase the moisture content of the finished cheese. When the curd is firmed, special rake paddles are substituted for the stirring paddles of the usual mechanical agitator. A half-round strainer is used to hold the curd away from the gate end of the vat. The whey is drained with practically continuous mechanical raking of the curd so that matting is prevented. Removing the whey requires about 30 min. by this process. Salt is applied from 30 to 45 min. after dipping ends and when the whey acidity approximates 0.30%. The whole process requires from 3 to 4 hr. from the time of renneting to hooping. W.V.P.

173. Grade and Composition of Swiss Cheese in Northwestern Wisconsin. C. B. LANE, Blue Moon Foods, Thorp, Wis. Natl. Butter and Cheese Jour., *38,* 3: 34. March, 1947.

Data on the composition of 19,642 wheels of Swiss (grade A, 4,046; grade B, 5,393; grade C, 4,899; grade open standard, 2,639; and grade standard, 2,665) obtained from 13 factories for a year beginning September, 1945, showed no practical correlation between composition (moisture and fat) and grade. W.V.P.

174. Factors Influencing Acid Production by Cheese Cultures. F. J. BABEL, Iowa Agr. Expt. Sta., Ames, Iowa. Natl. Butter and Cheese Jour., *38,* 2: 38. Feb., 1947.

This is an abridged discussion of the paper in the Journal of Dairy Science, *29*, 9: 597. 1946.

CHEMISTRY

175. The State of Vitamin A in Colostrum and in Milk. D. B. PARRISH, G. H. WISE, AND J. S. HUGHES, Kansas Agr. Expt. Sta., Manhattan, Kans. Jour. Biol. Chem., *167:* 673–678. 1947.

Vitamin A alcohol was separated from the vitamin A ester by the use of an alumina adsorption column, the ester passing through the column. Colostrum samples were taken within 4 hr. postpartum and milk samples from the same cows 3 to 8 weeks later. One Guernsey, 6 Jerseys, 7 Ayrshires, and 4 Holsteins were used in the study.

The authors conclude that: "Practically all of the Vitamin A in both colostrum and milk was found to be in the form of the ester." Preliminary studies indicated most of the vitamin A in the blood of these cows was in the form of alcohol. "Most of the fat-soluble yellow pigment in colostrum and in milk was found to be carotene." A.O.C.

176. Application of Sendroy's Iodometric Chloride Titration to Protein-Containing Fluids. D. D. VAN SLYKE AND ALMA HILLER, Hosp. Rockefeller Inst. for Med. Res., New York. Jour. Biol. Chem., *167:* 107–124. 1947.

The Sendroy iodometric method for chlorides is based upon the liberation and measurement of the IO_3 radical according to the following equation: $AgIO_3 + Cl^- = AgCl + IO_3^-$. The IO_3^- is measured by titrating against a standard thiosulfate solution.

"In the present application, the titrimetric procedure for protein-containing fluids is simplified by carrying out the reaction with $AgIO_3$ and precipitation of proteins simultaneously in a single operation, so that an entire analysis, including removal of the mixed precipitate and titration of the filtrate, can be carried through in about 6 minutes."

A table comparing this method and the nitric acid digestion method in the analysis of cows' milk is given. A.O.C.

177. The Determination of Iron in Biological Material. A. J. WOIWOD, Wellcome Physiological Research Laboratories, Beckenham, Kent (England). Biochem. Jour., *41,* 1: 39–41. 1947.

"A method for determining iron in biological materials with a high P : Fe ration is described. It is applicable over the range 0.5–10 μg. Fe/ml. All analytical manipulations, except the final centrifuging before colour reading, are performed in the crucible in which the sample has been ashed Blanks are therefore kept at a minimum.

"The method has proved satisfactory with protein hydrolysates and cows' milk and may be suitable for other materials where phosphate interference is met."

A value of 4.7 μg. Fe/10 ml. is reported for raw milk. Twelve references are given. A.O.C.

178. Constant Pressure Oxygen Absorption Fat Stability Test. G. GILMONT, H. S. LEVENSON, AND L. W. ELDER. General Foods Corp., Hoboken, N. J. Oil and Soap, *23,* 8: 248. Aug., 1946.

Details are given for the equipment and operation of the General Foods Method of determining fat stability. The method, essentially a modification of the Barcroft-Warburg procedure of Johnson and Frey, differs from the latter method in two respects: (a) The oxygen is absorbed under constant pressure and can be recorded volumetrically on a macro scale. (b) The induction periods can be evaluated graphically from the direct plot of the experimental data without further calculation. With a 2-ml. sample the induction periods were reproducible with a precision of 1 to 2% in most cases or a maximum variation of 5% in the most unfavorable cases.

J.L.H.

179. Interfacial Tension of Oil-Water Systems Containing Technical Mono- and Diglycerides. R. O. Fenge, So. Regional Res. Lab., New Orleans, La. Jour. Amer. Oil Chemists Soc., *24*, 2: 49. 1947.

Technical mono- and diglycerides have wide industrial use as oil-soluble emulsifying agents in the manufacture of superglycerinated shortenings and margarine. Commercially available products are composed of mixtures of mono-, di-, and triglycerides. Known mixtures of mono-, di-, and triglycerides have been studied but little with respect to their power of lowering the interfacial tension at vegetable oil-water interfaces. Technical mono- and diglycerides were prepared for study. When both mono- and diglycerides are present in the oil phase, the interfacial tension is substantially a function of the monoglyceride content. A constant weight of a given monoglyceride preparation had practically an equal effect in lowering the interfacial tensions against water for peanut, cottonseed, and soybean oils. A concentration of 1% of monoglyceride in the oil phase was found to lower the interfacial tension at the oil-water interface by approximately one-half, and 6% lowered the interfacial tension to practically zero.

J.L.H.

FEEDS AND FEEDING

180. Effect of Dehydration on Enzymic Destruction of Carotene in Alfalfa. H. L. Mitchell and H. H. King, Dept. of Chem., Kansas State College, Manhattan, Kans. Jour. Biol. Chem., *166:* 477–480. 1946.

As alfalfa cures in the field the enzyme lipoxidase is responsible for the destruction of a considerable part of the carotene. Peroxidase, another oxidative enzyme present in alfalfa, is inactivated by blanching prior to dehydration (mechanical drying). By blanching and dehydration both enzymes are inactivated and neither is regenerated during storage of alfalfa meal for 2 months.

"Blanching of alfalfa prior to dehydration did not increase the retention of carotene during storage. Carotene destruction during storage does not appear to be enzymic in nature."

A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

181. The Effect of Fat on the Absorption and Utilization of Galactose by the Rat. Marie L. Nieft and H. J. Deuel, Jr., Dept. of Biochem. and Nutr., Univ. of Southern California, School of Medicine, Los Angeles, Calif. Jour. Biol. Chem., *167:* 521–525. 1947.

The present work confirms, to a large degree, that reported by Geyer *et al.* (See Abs. 358, Jour. Dairy Sci., *29*, 10: A164. 1946.) The investigators conclude: "The percentage of ingested galactose which is excreted in the urine varies inversely with the percentage of fat in the diet."

"The fat effect appears to be independent of the type of fat at a 20 per cent level, since butter fat, corn oil, and cottonseed oil give essentially the same results. However, if the fat level is cut to 10 per cent, cotton-seed oil gives significantly lower urinary excretion values than either butter fat or corn oil." A.O.C.

ICE CREAM

182. Shrinkage in Ice Cream. C. D. DAHLE, D. J. HANKINSON, AND J. A. MEISER, JR. Ice Cream Rev., *30*, 6: 41. Jan., 1947.

The least amount of shrinkage was observed with ice cream stored in glass containers, followed in order by metal, paper paraffined inside and outside, paper paraffined on the inside only, paper paraffined on the outside only, and untreated paper. Shrinkage was greatly reduced when a cabinet temperature of +5° F. was used as compared with +10° F., irrespective of the type of container used. No shrinkage was observed in ice cream samples stored at −15 to −20° F. Substitution of 30% of the cane sugar on a dry weight basis with sweetose, cerelose, Frodex, or honey increased the amount of shrinkage in the order named. When corn sirup was substituted for 30% of the cane sugar, less shrinkage was observed than when cane sugar alone was used. Pasteurization of the mix at 160° F. for 20 min. or 160° F. for 45 min. resulted in slightly less shrinkage than when pasteurization temperatures of 145° or 175° F. were used. Wet (incompletely frozen) ice cream was found to shrink more than that frozen enough to yield a dry appearing ice cream when drawn from the freezer. High homogenization pressures were found to increase shrinkage, with little or no relationship being observed between the homogenization pressures used and the dryness of the ice cream at the freezer.

Four different stabilizers showed little difference in the amount of shrinkage. Certain emulsifiers and egg yolk increased the dryness of the ice cream and increased shrinkage somewhat. No relationship was observed between the source of gelatin nor its Bloom strength and shrinkage. The use of superheated condensed milk resulted in less shrinkage in ice cream than when plain condensed milk was used. Butterfat from different sources gave variable and inconsistent results as related to shrinkage. The mix acidity and protein stability could not be correlated definitely with shrinkage. The addition of sodium salts to ice cream mix had little or no effect on the amount of shrinkage observed. The addition of calcium salts did accentuate shrinkage slightly. Presence of 0.4% free fatty acids in the mix increased markedly the amount of shrinkage observed in the ice cream. The use of dry ice was found to accentuate shrinkage, particularly with ice cream frozen in a continuous freezer. W.J.C.

183. Manufacture of Powdered Ice Cream Mix. Harry Pyenson. Ice Cream Rev., *30,* 8: 54. 1947.

The composition, advantages, markets, and results of experimental studies dealing with the manufacture, storage, and use of powdered ice cream mix are discussed. Stabilizers of vegetable origin gave more satisfactory results than those of animal origin and improved the whipping properties of the mix when used in conjunction with an emulsifying agent, such as glycerol monostearate S. Addition of all of the sugar to the mix prior to condensing resulted in a caramel flavor, reduced the capacity of the drying equipment, and interfered with the drying process. The addition of only 25% of the sugar prior to condensing is recommended. High quality dairy products processed in stainless steel equipment, high pre-heating temperatures, presence of antioxidants and low moisture content (below 1.5%) were essential to the production of powdered ice cream mix of good keeping quality. Such a product showed very little change in flavor for 8 to 12 months or longer.

A suggested procedure for the production of powdered ice cream mix is as follows: (1) Combine fluid milk and cream to give the desired ratio of fat to solids-not-fat, condense, and add 25% of the sugar and stabilizer, or add the sugar and stabilizer to the milk and cream prior to condensing. (2) Preheat to not less than 170° F. for 20 min., 180° F. for 10 min., or 190° F. for 5 min. (3) Condense to 30–36% solids, depending on whether stabilizer is added before or after condensing. (4) Homogenize the condensed mix at 150° F., using a pressure of 2,500 lbs. for the first stage and 500 lbs. for the second stage. (5) Preheat the homogenized mix to 150° F. before spray drying. (6) Spray-dry the mix to yield a product to contain not over 1.5% moisture. (7) Cool the powdered mix immediately. This will minimize cooked flavor and retard the development of stale flavor. (8) Add the remainder of the sugar. (9) Gas pack the powder in tins.

Powdered mix usually analyzes: Butterfat, 27–30%; M.S.N.F., 27–28%; sugar, 39.5–44%; stabilizer, 0.6–1.0%; and unspecified material, 2.75%. The powder may be reconstituted by adding it to cold water in the freezer and freezing immediately, but better quality ice cream resulted when the reconstituted mix was allowed to age for 24 hr. prior to freezing. W.J.C.

184. Improving Package Ice Cream. J. H. Erb. Ice Cream Rev., *30,* 8: 44. March, 1947.

The challenge confronting the ice cream industry is to produce in factory-filled packages the same desirable characteristics the consumer associates with hand-dipped ice cream. Key factors in attaining this objective are: (1) Total solids content of the mix should be between 38.5–40%. (2) Use of high quality raw ingredients. (3) Thorough homogenization. (4)

Aging of mix for not less than 5 hr., irrespective of the stabilizer used. (5) Maintaining proper weight in all packaged ice cream. (6) Adoption of overrun standards well below those used for bulk ice cream. Freezers in battery operation should be checked to see that ice cream of the proper overrun is being delivered by each freezer. (7) Freezing the ice cream to a stiff dry consistency at the freezer and avoiding the use of long, small diameter lines in conducting the ice cream from the freezer to the filler head. (8) Hardening time not to exceed 6 to 8 hr. W.J.C.

185. "Sanitary Spoon Rest." ANONYMOUS. Ice Cream Rev., *30,* 8: 43. March, 1947.

Sales resistance to tall fountain drinks on the part of the customers who do not like to put wet spoons on the counter when not in use may be overcome through the use of an ingenious paper spoon holder known as "Sanitary Spoon Rest". This device, manufactured and introduced in the Los Angeles area by Coast Curries Ice Cream Co., helps keep the counters clean, prevents sloppy serving of the spoon, and also is an effective medium for advertising. W.J.C.

186. Lower Costs in the Ice Cream Industry. CARL H. ZANZOW, JR. Ice Cream Rev., *30,* 8: 48. March, 1947.

The objective of every ice cream plant to increase productivity and, simultaneously, reduce costs can best be achieved by the establishment of a complete production and engineering department, responsible only to top management. Functions of such a department are: supervisory training, plant layout and process improvement, work simplification and methods improvement, work measurement, employee training, planning and scheduling of operations, job evaluation, establishment of sound wage incentives, and cost control. A common understanding of the entire program is essential by all levels of management, the employees and their union, and can be achieved best by a series of group-participating conferences.

Results to be expected from a properly functioning production engineering program are: (1) proper plant layout and process flow, (2) simplified and standardized working methods, (3) establishment of fair and equitable work standards, (4) establishment of a fair and equitable base wage rate structure built upon a sound job evaluation procedure, (5) improved production scheduling throughout the plant, (6) increased employee earnings through the use of wage incentives, (7) reduction in unit costs by a greater output per man hour, (8) the control and elimination of material waste, (9) decreased labor turnover, (10) the establishment of standard costs, and (11) effective cost control reports to measure progress and point out weaknesses that can be remedied. W.J.C.

187. Trends in Ice Cream Merchandising. R. A. PERRY. Ice Cream Rev., *30,* 8: 45. March, 1947.

A program designed to surround ice cream with an atmosphere which will intensify its appeal and remove any barriers which might serve as obstacles to consumer acceptance must start with the manufacturer and carry through to the ultimate consumer.

The manufacturer has the responsibility of seeing that a uniformly high quality product, attractively packaged, is supplied at all times. The plant and delivery equipment must meet the most exacting demands of sanitation, orderliness, and attractiveness. The ice cream manufacturer must realize that he must follow his product through to the ultimate consumer. This involves closer cooperation between supplier and dealer. Sanitation at the point of sale is stressed as the phase of activity most deserving of immediate attention. The appearance of the store, its personnel, odors, cleanliness of the counter, seats, glasses, silverware, etc., all are factors which influence customer satisfaction.

Education of the dealer through actual visual demonstrations is presented as the most effective means of dealer education on the part of the ice cream manufacturer. Eleven concrete suggestions for making the dealer into an effective merchandiser for ice cream are presented. W.J.C.

188. Selling Ice Cream through Vending Machines. E. THOM. Ice Cream Rev., *30,* 7: 41. Feb., 1947.

The use of vending machines for the distribution of ice cream opens up a new and virtually untouched market. Experience gained since 1940 by Miller Bros. in New York City should prove extremely helpful to any ice cream company contemplating entering this field.

The operation of vending machines demands the full-time attention of a separate department or a separate company. It cannot be handled successfully as a part-time job on the part of some individual within the organization. The ice cream served must be of unvarying high quality which will merit repeat sales, and flavors should be confined primarily to vanilla and chocolate. The nickel ice cream cup is the item which probably can be sold most successfully through vending machines, although the possible vending of ten-cent cups and other novelty items is being tried out in a limited way. Vending machines with a capacity of not less than 200 cups should be installed and these must be kept supplied with ice cream at all times. Service men should be paid in part on a commission basis to increase their interest in keeping the machines filled and in operating order. They should not be expected to make repairs on machines except for minor adjustments requiring 10 min. or less. Vending machines should be located with firms primarily interested in providing a service for their employees rather than with firms interested in the commission which may be received as rental for the space and electricity provided. This method of distributing ice cream has the advantage of being a strictly cash business and serves to bolster sales during the winter months. W.J.C.

189. The Need for Cooperation. C. J. PALMER. Ice Cream Rev., *30*, 7: 48. Feb., 1947.

Cooperation between manufacturers of ice cream and of soda fountain equipment can do much to make soda fountain operators appreciate more fully the importance of good equipment and the importance of providing outstanding service to their customers. Such cooperation will result in increased sales of both ice cream and soda fountain equipment, thereby working to the mutual advantage of both groups. The two groups should cooperate to the end that: (1) Soda fountain operation will be above any possible criticism from a sanitary standpoint. (2) Proper facilities will be provided for the cleaning and sterilization of all multi-use utensils. (3) The possibilities of expanded food service at the fountain will be brought to the attention of the operators. (Surveys have shown that offering both food and fountain service will result in greatly increased ice cream and fountain sales as well as a more profitable over-all operation.) (4) Fountain drinks of superior quality will be served as a result of use of proper equipment and education of the operator in the essential steps of correct carbonation.
W.J.C.

190. The Importance of Ice Cream in the Dairy Industry. R. C. SMELLIE. Canad. Dairy and Ice Cream Jour., *26*, 3: 37. March, 1947.

The value of ice cream can be illustrated best by its continued manufacture during the war and at a volume in excess of the 1939 production. An increase in population and a decline in cow and heifer population are making less milk available for ice cream in Canada today. The dairy producers have to be convinced that all the milk they produce will have a profitable outlet. Ice cream is officially recognized as a food in Canada and more publicity will be given to it when more ice cream is available. H.P.

MILK

191. Milk Production Trends in the U.S.A. and Possible Competition from Canada. T. M. ADAMS. Canad. Dairy and Ice Cream Jour., *26*, 3: 59. March, 1947.

The sound position of dairying in the northeastern region of U.S.A. lies in the adaptability of the area to the economical production of an abundant supply of high quality roughage and pasture and its nearness to large populations of consumers of fluid milk. Cost of transportation and the quality factors associated with the movement of milk over long distances have made for an economic barrier excluding midwestern fluid milk from New England markets. Competition from Canada may increase if the barriers which limit milk imports are removed or lowered. H.P.

192. **Operation of Six-Day-Week Milk Delivery.** A. Giganac. Canad. Dairy and Ice Cream Jour., *26,* 3: 66. March, 1947.

Windsor, Ontario, has successfully operated on a 6-day-week milk delivery for the past 4 years. Milk is not delivered on Sunday or on a holiday that occurs on Wednesday or Thursday. The management, employees, and the public find 6-day delivery more economical and more pleasant, and it simplifies delivery operations. Six-day delivery and plant work are not difficult providing good storage facilities and excellent facilities for raw milk are available. H.P.

MISCELLANEOUS

193. **Pasteurization by Ultra-Violet Rays.** E. Capstick, H. Hall, and F. K. Neave. Milk Indus., *27:* 8. Feb., 1947.

The German Prof. Lembke and Dr. Bayha have developed a process for pasteurization by ultra-violet rays. The optimum wave length for a bactericidal effect is 2537 Å. The effective penetration is restricted to approximately 1 mm. A sheet metal cabinet houses 28 quartz-tube mercury vapor lamps. Milk flows through quartz tubes on a three-pass system to form a continuous path 328 feet long. The milk in each bank of 50 tubes is irradiated by 12 mercury vapor lamps. Air temperature in the cabinet is maintained at 20–30° C. The unit was designed to operate at 1,200 l. per hour (260 gallons) but operates at the present time at about 140 gallons per hour. The equipment is easily cleaned by circulating cold water, followed by a warm alkaline detergent solution, then water acidified slightly with HCl, and finally clear cold water. The bacteriological tests made show that an exposure for 1.75 min. with the milk at 30° C. gave better results than milk held for 30 min. at 63° C. With poor quality milk coliform organisms survived in the irradiated milk. Many more investigations have to be made to determine the proper amount of radiation, temperature of milk and lamps, turbulence, the importance of such factors as aeration and deaeration of the milk, and the effect of the process upon flavor, keeping quality, pathogens, and vitamin A. H.P.

194. **Safeguarding Your Water Supply.** N. P. Nupson, Pennsylvania Salt Mfg. Co., Philadelphia, Pa. Natl. Butter and Cheese Jour., *38,* 3: 42. March, 1947.

Bacterial contamination of the water supply may constitute a problem of health or quality control or both, depending upon the types of organisms present. Get rid of improperly constructed wells, keep water supply tanks clean, and eliminate dead-end piping. If contamination exists, then the water coming in contact with the food can be pasteurized, the well itself may be chlorinated, the water supply tank can be chlorinated (usually 5 to 10 p.p.m. is adequate dosage), or a device can be used to inject hypochlorite solution continuously into the water line. W.V.P.

195. Saving and Disposal of Creamery Waste.

O. W. SANDBORG, Armour & Co., AND A. J. STEFFEN, Wilson & Co., Chicago, Ill. Natl. Butter and Cheese Jour., *38,* 2: 34. Feb., 1947.

Improper disposal of dairy wastes can cause justified complaints and law suits. Dairy wastes are caused by inefficient equipment, methods, or operations. They may consist of buttermilk, skim milk, other by-products, rinsings, and wash water. The wastes can be reduced by using drippings from emptied cans for animal feed, using automatic controls where milk may overflow, preventing leaks, educating personnel to avoid spilling and splashing of products, and using all by-products. The system of waste disposal to be employed can be determined only by careful study of waste flow, waste concentration, proximity to a flowing stream, and relation to city sewage disposal. Frequent waste sampling and testing plus the determination of the causes of excessive losses are essential for loss reduction. W.V.P.

196. Booster Compressors.

BERNARD SAVEY. Ice Cream Rev., *30,* 8: 52. March, 1947.

Booster compressors are suggested as the solution in adapting present ice cream refrigeration systems to fit the needs of the modern ice cream factory. Boosters are described as compressors which pump the gas from freezers, hardening rooms, and other low temperature rooms through water cooled and gas cooled coolers into the conventional or second stage compressors, which, in turn, compress and discharge the gas into condensers.

Advantages claimed for such a system are lower initial cost, maintenance of constant low temperatures unaffected by other plant loads, lower operating cost due to precooling of liquid and intercooling of low pressure gases, and less ammonia condenser cooling water required due to less total heat to be removed from the high pressure gas.

Although conventional type compressors may be used as boosters, boosters designed for this purpose are more economical. They are lighter in weight and have less bearing surface between the pistons and cylinder to contribute to friction losses. W.J.C.

197. **Refrigerating Oil Carryover at High Temperatures.** J. M. LEBEAUX, AND LUIS H. BARTLETT, Univ. of Texas. Refrig. Engin., *53*, 3: 203–207. March, 1947.

Experimental results on oil carryover were obtained for 15 oil samples by means of a specially designed test flask. It was proved conclusively that refrigeration oils vaporize at the discharge temperatures of the compressor. The amount of oil carryover increased considerably with an increase in temperature, the pressure being held approximately constant. The greatest carryover occurred with most of the lighter oils. However, the heavier oils with lower carryover are unsuited to small high-speed installations. On the other hand, if the oil can be excluded from the evaporator, the pour test is a matter of little consequence. The oil also should have either a negligible solubility or complete solubility in the refrigerant. The most suitable refrigerating lubricants are petroleum oils of paraffinic and napthenic types; the latter, due to their inherent freedom from wax and resultant low pour test, are in general use throughout the refrigerating industry. The napthenic oils are mandatory where sulfur dioxide is used as a refrigerant but are equally satisfactory with the other common refrigerants. Heavier oils are called for in large machines for the heavier duty requirements or for refrigerants that have a high miscibility or mutual solubility with oil. Rotary type compressors require heavier oils than reciprocating or piston type.

The precise selection of the best lubricating oil will depend upon the design, capacity, operating conditions, and the type of the refrigerant used, and should be governed also by the vapor pressure of the oil to insure a minimum carryover and hence a maximum efficiency of the system.

L.M.D.

198. **Air Blast Quick Freezing.** REGIS GUBSER, California Consumers Corp. Refrig. Engin., *53*, 1: 23. Jan., 1947.

The essential features of air blast freezing systems are presented briefly. Air movement at the rate of 1500 c.f.m. per ton of refrigeration application is required. The maximum performance of a freezing tunnel is obtained when the air passages are kept small around the product and around the coil surface. The higher the velocity the greater the K factor. In no case should the velocity be less than 500 ft./min., and it should be as much above this rate as will be permitted by the friction loss and the static pressure developed by the fan. L.M.D.

199. **Frozen Cooked Foods.** FAITH FENTON, State College of Home Economics and School of Nutrition, Cornell University. Refrig. Engin., *53*, 2: 107–111. Feb., 1947.

Details of carefully controlled laboratory procedures for freezing certain foods, including custard-base ice creams, are reported. Results favored the freezing of raw yeast rolls and unbaked pies and their subsequent baking after removal from freezer storage. Rolls made with milk scored higher in flavor and moisture than did those made with water. Ice creams frozen in a hand freezer and hardened in the various parts of the freezer cabinet were superior in smoothness. Ice cream frozen in the ice cube compartment, which ranged from 13 to 15° F., was smoother than that frozen in the freezer compartment, −20° F., or in the storage space, −5° F., the latter producing the coarser ice crystallization. The quicker freezing in the ice cube section, with the bottom of the tray containing the ice cream being in direct contact with the freezing plate, indicated that the rate of freezing was more important than the environmental temperature, even with forced convection in the freezer compartment. Ice creams developing coarse ice crystallization while freezing became increasingly coarse with prolonged storage. L.M.D.

200. A Qualitative Method for Detecting Surface Active Agents. L. F. HOYT, National Aniline Div., Allred Chemical and Dye Corp., Buffalo, N. Y. Jour. Amer. Oil Chemists Soc., *24,* 2: 54. Feb., 1947.

A new method, consisting of the solubilizing of a Brilliant Oil Blue B.M.A. solution to produce a blue solution, is described for the qualitative detection of small amounts of surface active agents. The method is applicable to all types of surface active agents (*i.e.*, anionic, cationic, and nonionic) and to dry, liquid, or paste surfaces. Fifty agents have been tested. J.L.H.

201. World Food Outlook and the Dairy Industry. GOVE HAMBIDGE, Canad. Dairy and Ice Cream Jour., *26,* 3: 25. March, 1947.

The work of the Food and Agricultural Organization of the United Nations is solving the widespread food shortage on a health standard for all people. The article discusses the dangers of over-production, the world food board, the Bruce commission to study proposals and make recommendations to the Food and Agricultural Organization, the high increase in production, and markets in backward lands. H.P.

202. Public Relations. J. W. LAWRENCE. Canad. Dairy and Ice Cream Jour., *26,* 3: 33–36. March, 1947.

Public relations begin at home, and your own staff must be sold on the company before you can sell the public on it. The employees are partners as well as consumers in the industry. The producers' viewpoints must be recognized. Individuals and companies should work with their competitors on the same major problems. The dairy, in general, works for the interests of the public, and consumer education should not be neglected. Profits and wages are the lubricant of industry. Publicity is only one of the tools of public relations. H.P.

203. **The Need for Apprentice Training in the Dairy Industry.** W. H. SPROULE. Canad. Dairy and Ice Cream Jour., *26*, 2: 28–30. Feb., 1947.

A well-formulated apprenticeship scheme can aid greatly in developing trained workers to provide leadership to the industry in the years to come. H.P.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

204. Coliform Bacterial Contamination of Milk and Milk Products. R. P. MYERS, Natl. Dairy Products Corp., Research Laboratories, Baltimore, Md. Milk Dealer, *36,* 6: 150–153. March, 1947.

The following sources of coliform contamination are discussed: (1) improperly sterilized equipment; (2) contamination of equipment when assembling it after sterilization; (3) condensate dripping from pipes, valve handles, ceilings, vat covers, cooler covers, and ice cream freezers; (4) handling any piece of equipment which comes in contact with pasteurized dairy products; (5) air contamination; (6) contamination of pasteurized milk with improperly pasteurized milk; (7) vacuum lines at the bottle fillers; (8) in the case of ice cream, the addition of contaminated colors, flavors, and fruits; (9) rubber gaskets and packing glands; (10) dead-end tees, connecting pipes between headers and vats, the upper part of horizontal lines, valves, and joints in pipelines; and (11) seepage through plug caps from contaminated water.

Coliform bacteria grow surprisingly well at relatively low temperatures, *i.e.,* 45 to 50° F., and therefore the coliform test is of greatest value only on freshly processed samples. The control of coliform contamination depends upon care and precision in carrying out the cleaning and sterilizing operations. C.J.B.

205. A Survey of the Direct Microscopic Method of Examining Milk and Cream Samples in Approved and Registered Laboratories of Connecticut. R. EGLINTON, State Dept. of Health, Hartford 1, Conn. Jour. Milk Technol., *10,* 1: 27–29, 33. Jan.–Feb., 1947.

A survey of the methods used in 14 approved and registered laboratories in Connecticut reveals a variation in the results obtained because the standard procedure was not followed uniformly in the different laboratories. Some of the obvious irregularities in connection with the use of the direct microscopic examination of the milk were: (1) Samples of milk were not collected in an approved manner; in some instances Babcock milk sampling dippers were used to collect the milk for bacteriological examination without any attempt to sterilize the sampling equipment. (2) Milk smears were examined by microscopes without a known factor. Microscopic findings may be used more effectively if a prompt follow-up of the laboratory examination is made by the field inspectors. H.H.W.

206. Ultraviolet in the Dairy Industry. A. J. DUSAULT, Westinghouse Electric Corp., Bloomfield, N. J. Milk Dealer, *36,* 6: 49, 100–102. March, 1947.

The bacteria count in the barn, milk and cooling rooms can be reduced as much as 94% by irradiating the atmosphere with bactericidal ultraviolet. When applied locally to washed milk cans and bottles, the bacteria count may be cut 96%; when used to irradiate the milk flowing over a surface cooler, a reduction in bacteria of 50 to 90% results, depending on the thickness of milk film and rate of flow. Surface mold on cheese is prevented by irradiating the curing room, and taste and quality of ice cream are improved by processing and packaging in a sterile atmosphere.

There is a definite place, if not an urgent need, for ultraviolet in the dairy industry. It performs a function entirely separate from existing sanitary procedures in that it disinfects the air, which heretofore has not been possible by means of steam or chemicals. Therefore, the combination of bacteria-killing ultraviolet, plus good sanitation, will insure both the producer and consumer of the finest quality product obtainable. C.J.B.

CHEESE

207. Causes of Off-flavors in Cheese. J. M. BAIN. Canad. Dairy and Ice Cream Jour., *26,* 4: 60. April, 1947.

Off-flavor has been the cause of 77% of the second grade cheese. The prevalent off-flavors are slight rancid, fruity, and not clean. Rancid flavors are most prevalent in the spring and fall, indicating that the milk supply (cows in early or late lactation) is responsible. Fruity flavor comes from unsatisfactory factory conditions such as improper pasteurization of whey, dirty pipe lines, and leaking vats. Other causes for fruity and unclean conditions are dirty milking machines, unclean utensils, improper washing of cans, and feed flavors. The cleanliness of Canadian cheese has improved markedly in the past 2 years. H.P.

208. Canadian Research Develops New Cheese. ANONYMOUS. Canad. Dairy and Ice Cream Jour., *26,* 4: 35. April, 1947.

"Blufort", a combination of roquefort and Danish Blue cheese, has been developed in Canada. A new type of quick-acting *Penicillium roqueforti* is used under controlled conditions of temperature, humidity, and ventilation. The cheese is not ripened in caves but in carefully controlled rooms at a temperature of 42–48° F. and a humidity of 90%. The cheese is kept for several months to develop the proper mold growth and flavor. H.P.

209. Extraneous and Other Matters. A. L. GIBSON. Canad. Dairy and Ice Cream Jour., *26,* 4: 41. April, 1947.

The detection of extraneous materials in cheese has led to improved methods of manufacture, but still only 3.4% of cheese is designated as no. 1 clean. The two main sources of extraneous matter in cheese are: (1) during the production of the milk on the farm and delivery to the factory, and (2) during the process of manufacturing the cheese. About 95% of the contamination is in the milk before delivery. The ultimate remedy lies in clean milk production coupled with perfection of manufacture. Straining milk should be looked upon as a final precaution to remove accidental contamination rather than a method of cleaning milk. H.P.

210. The Influence of Acidity on the Development of Lipolytic Flavour Defects in Cheddar Cheese.

I. Hlynka, E. G. Hood, and C. A. Gibson, Dominion Dept. of Agr., Ottawa, Canada. Sci. Agr., *27*, 1: 50–56. Jan., 1947.

Earlier studies had shown that agitation of cheese milk produced rancid and unclean flavors in raw milk Cheddar cheese. The results secured when acidity was employed as an inhibitory agent are given, the results being assessed as flavor scores. Additions of hydrochloric acid as a means of acidification were only partially successful. Manufacturing methods involving the use of larger amounts of starter or longer periods of milk ripening were beneficial. In comparative experiments where lipase activity was induced in both vats by homogenization, flavor scores were 1 to 1.5 points higher. The results are explained on the basis of lipase inhibition by contact with acid over a period of time, as well as by influence of acid which affects lipase action in such a way as to produce mainly non-volatile acids.

O.R.I.

CHEMISTRY

211. Determination of the Titratable Acidity of Milk.

E. H. Zilliox, D. J. Mitchell, and G. G. Frary, State Chemical Lab., Vermillion, S. Dak. Assoc. Off. Agr. Chem. Jour., *30*, 1: 130–134. 1947.

Since lactic acid is practically absent from fresh milk, use of a dilution of the milk and an amount of indicator which would give the lowest apparent acidity seemed logical. An increase in the amount of phenolphthalein indicator (1% in 95% alcohol) from 0.5 ml. to 2.5 ml. decreased the pH of the solution at the end point from pH 8.25 to 8.02; larger amounts of indicator did not change the pH at the end point. The concentration of phenolphthalein indicator did not affect the end point in a water solution of pure lactic acid. When 2 ml. of phenolphthalein was used as indicator, the volume of alkali needed to reach the end point decreased as dilution of the milk was increased; a sample of undiluted milk required 2.89 ml. of 0.1 *N* sodium hydroxide while the same milk diluted 1 + 10 required 1.75 ml. A 1 + 2 dilution of the milk required about the same amount of alkali with either 2 ml. or 3 ml. of

indicator. When 2 ml. of indicator was used, a dilution of the milk greater than 1 + 2 did not affect the results significantly. The authors recommend that 17.6 ml. of milk be diluted with twice its volume of carbon dioxide-free water and titrated with 0.1 *N* sodium hydroxide to the first definite pink color, using as indicator 1 ml. of 2% phenolphthalein in neutral alcohol. A sample of milk having a titratable acidity of 0.135% by the present official method would show a titratable acidity of 0.110 by the method suggested.
F.J.B.

212. Contribution a l'Étude de la Methode Kohler pour la Dosage de la Matiere Grasse des Crémes (Contribution to the Study of the Kohler Method for Determining Fat Content of Cream). C. HUREL AND G. MACQUOT. Le Lait, *26*, 259–260: 289–301. Oct.–Dec., 1946.

Details are given of the methods used in determining the composition and properties of the material separated as fat in butyrometer tubes when cream is tested by the Kohler (similar to Gerber) test. Results indicate that this material is 0.9 to 1% amyl alcohol, 0.7 to 0.8% sulfuric acid, and 98.2 to 98.3% fat. The actual density of the material by pycnometer was between 0.8838 and 0.8860. The degree of extraction of the method was found to be 99%.
O.R.I.

213. Determination of Copper in Foods by a Two-color Dithizone Method. C. A. GREENLEAF, Natl. Canners Assoc., Washington, D. C. Assoc. Off. Agr. Chem. Jour., *30*, 1: 144–152. 1947.

An all-dithizone two-color micro method for copper is proposed. Distinguishing features of the test are the use of variable strengths of dithizone in proportion to the amount of copper in the final determination and the use of permanent calibration based on a method of optical density at two wave lengths. Preliminary results indicate that the method is free from interferences by other metals and that it gives good accuracy and precision.
F.J.B.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

214. Dried Milk Powder. VII. The Effect of Season of Production on Keeping Quality. J. A. PEARCE AND W. A. BRYCE, Natl. Research Laboratories, Ottawa, Canada. Jour. Res., *25*, F., 1: 13–17. Jan., 1947.

Palatability scores on skim milk powders produced in early December were higher than on similar powders produced in late May. Powders from milk produced in the fall decreased in quality when stored for 16 weeks at temperatures of 80, 100, and 120° F. Skim milk powders produced during spring improved throughout storage at 80° F., while those stored at 100 and 120° F. first increased and then decreased in quality. Powders of 26 and 28% butterfat produced in fall or in the spring had equal initial palatability scores and, when stored, deteriorated equally. Fall powders with 30% butterfat had higher initial scores but also deteriorated. Powders were packed in tin-plate containers with air as the headspace gas. The results are discussed in the light of seasonal changes in milk composition. O.R.I.

FEEDS AND FEEDING

215. Pasture Management for the Prevention of Onion Flavor in Milk.

C. E. WYLIE AND S. A. HINTON, Dairy Dept., Univ. of Tennessee, Knoxville. Tenn. Agr. Ext. Service Leaflet 89, 4 pp. 1947.

Various methods of controlling onion flavor in milk are discussed. The practice of removing cows from onion pasture 3 or 4 hr. before milking not always is effective. In early spring the contaminated pasture first may be grazed by dry cows and heifers until there is no new growth of onion tops. A period of 10–14 days should intervene to permit the pasture to get started again. Clipping onion tops with a mowing machine after new growth stops has proved successful in preventing onion flavor in late spring but is not satisfactory once the onions start growing again. Dried onion tops or cured hay containing tops produced no undesirable flavors. Eradication, where possible, is more desirable than any other means of control. F.E.N.

FOOD VALUE OF DAIRY PRODUCTS

216. Feeding of Premature Infants—A Comparison of Human and Cow's Milk.

H. H. GORDON, Denver, AND S. Z. LEVINE AND HELEN MCNAMARA, New York, New York Hosp. and Dept. of Ped., Cornell Univ. Medical College, N. Y. C. Amer. Jour. Dis. Children, *73,* 4: 442. April, 1947.

Studies of weight gains of premature infants from the 7th to the 28th day of life indicated that cow's milk mixtures are better suited than human milk to the peculiar needs of small premature infants. The superiority of cow's milk was thought due to its higher protein content.

Average gains in weight for the 122 infants studied were 12.5 g. per kg. per day for those fed human milk, 14.1 g. for those fed an evaporated milk mixture, and 15.7 g. for the infants fed half-skimmed milk. When the infants were divided into two groups according to whether they were above or below the mean birth weight of 1621 g. (3 lb., 9 oz.), the chief differences in weight gains were found in the smaller prematures. For those infants with birth weights above 3 lb. 9 oz., the differences in weight gains on the three types of milk were not statistically significant. However, in the small prematures, the differences between human milk and the cow's milk mixtures were more pronounced. Mean gains in weight per kilogram per day were 11.7 g. for the small prematures fed human milk, 14.9 g. for those fed evaporated milk mixtures, and 17.3 g. for those fed half-skimmed milk. M.B.

217. **Availability of Vitamins in Foods and Food Products. III. Riboflavin Balances with Liquid Milk, Dried Skimmed Milk, and Evaporated Milk and the Influence of Succinyl Sulfathiazole on Such Balances.** B. SURE, Dept. of Agr. Chem., Univ. of Arkansas, Fayetteville. Arch. of Biochem., *12,* 3: 389. 1947.

Riboflavin balance studies on rats fed liquid cow's milk, dried skimmed milk, and evaporated milk indicated a good utilization of riboflavin from all these sources. Larger fecal excretions of riboflavin in rats which obtained their riboflavin from milk as compared to rats which received the pure vitamin were attributed to intestinal synthesis of the vitamin in rats on the milk diet. Upon the introduction of 2% succinyl sulfathiazole in the diet of the rats, a marked decrease in fecal riboflavin was noted.

The rats which received their riboflavin in milk also excreted more riboflavin in the urine than those which received the pure vitamin. This was due to an elimination of excess vitamin, rather than poor utilization, because the rats receiving riboflavin in milk gained about twice as much weight as the others. Also, weanling rats whose stores of riboflavin had been depleted gained weight as well when given 250 mg. dried skimmed milk furnishing 5 μg. of riboflavin daily as when given 5 μg. daily of the pure vitamin.

M.B.

HERD MANAGEMENT

218. **Managed Milking.** I. E. PARKIN, Pennsylvania State College. Milk Dealer, *36,* 6: 68–70. March, 1947.

Managed milking is defined as the common sense practice of controlling or managing the milking of the cow when you want to milk, rather than having the cow manage the milking when she wants to be milked.

After discussing the milk manufacturing process, the author discusses the milking process. During the approximate time of 3 min. in which most cows are milked, the three things that must be done are the preparation of the cow, the disposal of milk, and the machine stripping of the cow.

C.J.B.

ICE CREAM

219. **High Temperature Vacuum Processing of Ice Cream Mix.** I. N. HAGEN, I. N. Hagen Ice Cream Co., Uniontown, Pa. Ice Cream Rev., *30,* 10: 144. May, 1947.

Six years commercial experience with the Vacreator has indicated that vacreation will produce ice cream mix of superior flavor and a mix with a lower viscosity than that processed in the conventional manner. The high temperature treatment under a partial vacuum has proved effective in removing undesirable feed and weed flavors and in eliminating oxidized flavors. The fine natural cream flavor is retained in the vacreated mix. Disadvantages of using the Vacreator are: (1) additional investment in equipment, (2) greater steam requirements for operation of the Vacreator as compared to the conventional method of pasteurizing ice cream mix, (3) extra time involved in cleaning the equipment, and (4) the absence of accurate and constant temperature controls required by many state health authorities. W.J.C.

220. **Fast-Freezing Tunnels.** H. Bitter, Wm. Neilson Ltd., Toronto, Canada. Ice Cream Trade Jour., *43,* 3: 50. March, 1947.

Ice cream packaged directly from the freezers must be hardened at a much faster pace to keep up with the freezers. A tunnel in which a temperature of at least – 40° F. is maintained and a blast of air is circulated over the package conveyor provides fast freezing. Tunnels are equipped with four 30-in. high-speed fans. The air is forced through the coil by the two front fans and through the tunnel and over the conveyor by the two back fans. This method takes 70 min. to harden the pint packages ready for shipment, at a capacity of 375 gallons per hour. The conveyor can be adjusted to travel the length of the 80-foot tunnel in from ¾ to 4½ hr.

The low temperature tunnel has several disadvantages. At – 40° F. the oil in the bearings and transmission is very likely to solidify. If this happens, the oil must be melted with blow torches. Oil and kerosene mixed in about equal parts is used, and even this mixture is not safe at – 50° F. With high velocity fans circulating the air in the tunnel, cold air is blown out the discharge port and warm air drawn into the intake. Even a small leak causes a loss in refrigeration and a formation of ice around the conveyor that soon builds up enough to interfere with its operation. A fan mounted inside the freezer, blowing against the air current trying to escape, can be adjusted so that no draft is present either at the intake or exit ports, and the formation of frost inside the tunnel, with the resulting refrigeration loss, can be held to a minimum.

While it is possible to use temperatures lower than – 40° F., the added cost does not appear to be justified if the tunnel is long enough to harden at the capacity of the freezers. Products should be stored in individual packages so there is no dead air insulating them. If Dixie cups are packed 12 in the box, very little hardening is accomplished in 1 hr.; if the lid of the box is left open, they will harden completely in 45 min.

Defrosting is best accomplished by the use of hot gas in as short a time as possible. If it is done by allowing the temperature of the room to rise, the change in temperature may cause the cork layer to separate and crack. Defrosting twice a year is ample. W.H.M.

221. Ice Cream Shrinkage, Flavor, Color and Packaging. C. A. IVERSON. Canad. Dairy and Ice Cream Jour., *26,* 4: 31. April, 1947.

Ice cream shrinkage can be minimized by: (1) using superheated condensed milk; (2) using corn sirup instead of corn sugar; (3) avoiding enzyme improvers and sodium alginate; (4) reducing milk solids and sugar; (5) maintaining a higher draining temperature at the freezer; (6) controlling overrun and keeping overrun under 85%; (7) keeping ice cream at a uniformly low temperature; (8) avoiding melting at the surface; (9) using metal rather than paper containers; and (10) avoiding packing in dry ice and later allowing to warm up. The color of ice cream should harmonize with the flavor. Colors are perishable and can contaminate the mix if not made and kept properly. Off-colored ice cream may be produced by colors that act as indicators. Packaged ice cream should have uniform overrun not to exceed 75%, should be frozen quickly after filling, should be kept at a lower temperature than bulk ice cream, and should have a rapid turnover. H.P.

222. Ice Cream Production Problems. C. A. IVERSON, Iowa State College, Ames. Ice Cream Rev., *30,* 9: 41. April, 1947.

Flavor, a key factor in consumer acceptance of ice cream, can be improved through the use of better quality dairy products, better quality of added flavors, and by avoiding the development of off flavors. The use of the vacuum pan or Vacreator is suggested as a means of enhancing the effect of fine quality vanilla. Color of the correct shade and intensity will enhance the eye appeal of ice cream, whereas the use of too much color, variations in color, or the wrong shade of color for the flavor represented will detract from its appearance.

Low overrun (not over 75%), uniformity of overrun, avoidance of partial melting, rapid turnover, and low cabinet storage temperature are important factors in the successful production and handling of packaged ice cream. Suggestions offered for the possible control of shrinkage include use of high quality milk products, use of superheated condensed milk, reduction in the solids content of the mix, use of a higher drawing temperature, reduction in overrun, and elimination of the use of enzyme ice cream improvers. W.J.C.

223. The A & P Spotlights Ice Cream. V. M. RABUFFO. Ice Cream Trade Jour., *43,* 2: 34. Feb., 1947.

A & P is manufacturing ice cream at the evaporated milk plant at West Bend, Wis., and merchandising it through some of its super-markets in Milwaukee. The ice cream, sold under the brand name of "Holly Carter", is reported to contain 14% fat and 41% total food solids, with an average overrun of about 45%. Pints retail for 35 cents, quarts for 70 cents, and half-gallons for $1.35. Vanilla, chocolate, and strawberry ice cream are packed in tray-pack, Philadelphia square, and round shaped containers in an attempt to determine consumer preference for package type. An attempt also is being made to determine the best location in the store for the dispensing cabinet.

Window posters were used to announce the new ice cream, and trained demonstrators invited customers to sample the ice cream. The Jiffy bag and proper amount of dry ice are included in the purchase price, and the customer is instructed on how to handle the dry ice and when it should be removed from the bag. Folders describing the new ice cream and recipes for serving are distributed. During December sales were reported to be from 50 to 75 gallons per store, with most of the sales on Friday and Saturday. W.H.M.

224. **Food Store Opportunities for Ice Cream.** V. M. RABUFFO. Ice Cream Trade Jour., *43*, 2: 36. Feb., 1947.

The 350,000 retail grocery outlets in this country, both chains and independents, are potential handlers of ice cream. These stores are the logical channel in the distribution system for getting food into the home and could increase the volume of sales appreciably. Firms preferring not to sell to the chains and other food stores need to make present dealers into better merchandisers of ice cream. Present dealers may have to take a more realistic mark-up on ice cream if they are to compete with the food stores. Some two million new household refrigerators were built in 1946 and about four million will be built this year. All of these new refrigerators will have low temperature storage compartments for ice cream and frozen food. Also, thousands of home freezers will be available for storing ice cream. W.H.M.

225. **The Future of Packages.** V. M. RABUFFO. Ice Cream Trade Jour., *43*, 3: 46. March, 1947.

Factors which will make possible a greatly expanded sale of ice cream in factory-filled packages are: (1) the introduction of packaged ice cream into the nation's food and grocery stores; (2) the new household refrigerator with its low temperature storage compartment providing a place for keeping ice cream in packaged form in the home; (3) the additional facilities for storing ice cream, provided by home and farm freezers, making ice cream available for every-day consumption by even the farm group; (4) the introduction of new equipment for the completely automatic packaging of ice cream, which will mean faster production and a lowering in production costs; (5) the introduction of larger package units such as the half-gallon and the gallon containers for home use; (6) the results of the widespread research on new types of packaging, packaging materials, new designs, etc., in connection with ice cream packages; (7) the relationship of ice cream with frozen foods in the home and at the dealer outlet, ice cream being the first frozen food; and (8) lower overrun and other assets in dealer analysis of the future market for packaged ice cream.

Other factors influencing the future of ice cream packaging are the tying in of ice cream with milk deliveries to the home, and the sale of ice cream by department stores who have set up frozen foods departments, by frozen food distributors to retail grocery stores, and by house-to-house sales organizations in the bakery, meat, and frozen food trades. W.H.M.

226. **Gallonage at Peak.** ANONYMOUS. Ice Cream Trade Jour., *43,* 2: 42. Feb., 1947.

According to official estimates made by the U. S. Department of Agriculture, the 1946 gallonage of ice cream hit the unprecedented total of 740,470,000 gallons, an increase of 55.9% over the 1945 volume. December production was about 80% above the 1940 to 1944 average. W.H.M.

MILK

227. **Increasing Homogenizer Capacity in the Processing of Homogenized Bottled Milk.** F. J. DOAN, Pennsylvania Agr. Expt. Sta., State College. Milk Dealer, *36,* 7: 47, 48, 94–96. April, 1947.

A method of increasing homogenizer capacity is discussed. A highly satisfactory homogenized milk can be produced by first separating the fluid milk and homogenizing only the cream portion, at a low fat concentration of about 10 to 12%. The processed cream then is recombined with the skim milk and the reconstituted milk finally pasteurized. The use of cream with too high a fat content will result in clumps of the small fat globules upon homogenization, and these in turn will contribute to instability of the fat emulsion of the final product.

The method will increase enormously the capacity of homogenizing equipment and may, where the homogenizer is entirely too small, help considerably in making the general plant operation more efficient. C.J.B.

228. **Safety Factors of H. T. S. T. Pasteurizers.** C. W. WEBER, New York State Dept. of Health, Albany, N. Y. Jour. Milk Technol., *10,* 1: 14–26. Jan.–Feb., 1947.

A critical appraisal of high temperature pasteurization of milk revealed a wide variation in the temperature to which the milk was exposed. A temperature of 160° F. for several seconds often was considered effective in destroying the organisms in the milk. However, health officials believed that safe milk could be produced if standards were adopted which specified that every portion of milk be heated to 160° F. or higher and held continuously for 15 seconds or longer. From the information now available, heating milk to 160° F. and holding for 6 seconds or less is sufficient to destroy pathogenic organisms in a milk supply. A 15-second holding time assures a safety zone of 9 seconds or more. When the holding time is 16 seconds, the actual minimum holding time will seldom be less than 15 seconds.

Raw milk may by-pass the pasteurizer through leakage of a defective milk-to-milk regenerator. However, this is not a serious defect if the pasteurizer is properly designed and operated so that the raw milk always is under less pressure than the pressure on the pasteurized milk side of the regenerator. H.H.W.

229. **Quality Control of Milk in the Pasteurizing Plant.** W. A. Josephson, Sheffield Farms Co., N. Y. C. Milk Dealer, *36,* 6: 60, 61. March, 1947.

To insure a continuous high quality product, the following essentials, stated in the order of their importance, must be provided: (1) a sincere belief on the part of all members of management that quality always is the first consideration; (2) standard practices stated, preferably in manuals; (3) operating personnel completely familiar with the foregoing standard practices; and (4) laboratory facilities and personnel adequate to make necessary measurements of product and process quality to guide management in its quality "control" activity. C.J.B.

230. **Production et Controle Hygienique du Lait dans les Étables Urbaines et Suburbaines** (Production and Sanitary Control of Milk in Urban and Suburban Stables). A. Louis. Le Lait, *26,* 251–253: 1–16; 254–256: 125–142; 257–258: 221. 1946.

The production of milk of superior hygienic quality at points close to the consumer presents special problems because of year-round stabling, prevention of disease, etc. In an extensive review the author describes the following phases of milk production: proper stable and milk house construction, the maintenance of herd health with particular reference being given to tuberculosis and mastitis control, the health of dairy personnel, sanitary methods of milking, proper ingredients in the ration to maintain herd health, and grooming of the animals. O.R.I.

231. **What's Ahead for the Fluid Milk Industry.** J. O. Eastlack, The Borden Co. Milk Dealer, *36,* 6: 130–134. March, 1947.

Following a discussion of the production, consumption, and price changes of milk, the author predicts that: (1) the less-frequent delivery systems adopted during the war are here to stay and 3-times-a-week delivery may come; (2) complete adoption of the square bottle during the next few years; (3) short-time high-temperature pasteurization will replace the holding method; (4) increased acceptance of homogenized milk; and (5) possibly all of the Northeast will look to the Midwest for its cream supply. C.J.B.

232. **Mid-Western or Canadian Milk for New England?** T. M. ADAMS, Univ. of Vermont. Milk Dealer, *36,* 7: 134–137. April, 1947.

See Abs. 191, Jour. Dairy Sci., *30,* 6: A85. June, 1947.

233. **Expanding Markets for Milk and Its Products.** R. W. BARTLETT, Univ. of Illinois, Urbana. Milk Dealer, *36,* 7: 150–152. April, 1947.

Opportunity now exists for expanding markets for dairy products by lowering distribution costs, by developing new techniques for dispensing milk to consumers, and by getting people to use more dairy products by popularizing known facts about the energy-building and nutritive value of these products. Some factors pertinent to the problem are discussed.

C.J.B.

234. **Trends of the Milk Industry.** J. J. SNYDER, Pennsylvania Milk Control Commission. Milk Dealer, *36,* 7: 153–155. April, 1947.

A discussion of the trends of the milk industry is summarized as follows: (1) The present high prices paid producers per hundredweight for milk eventually will bring a plentiful if not a burdensome supply of milk in the spring. (2) Fall shortages will continue to be acute in the absence of a decided business recession for at least 2 or 3 years. (3) The acuteness of fall shortage can be minimized greatly by educational programs fostered by producers' and dealers' associations. Special attention should be called to the fact that artificial insemination is the best practical way to improve the breed, produce milk cheaply, and achieve level production. (4) Dairy products must be priced fairly all the way through to consumers and at no point should they have to bear undue cost of distribution. Both producers and distributors must give careful attention to the law of diminishing returns. (5) The price of milk, especially fluid milk which bears little connection with pricing manufactured milk, if placed on a formula, must be on a formula in which the factors cannot be manipulated by any group or groups. Educational programs in nutrition and the support of educational programs fostered by co-operatives and dealers' associations must receive more attention and more whole-hearted support, both morally and financially. (6) The drive to effect economies in distribution must continue unabated. The time has come when producers and dealers no longer can remain static in production and distribution costs and rely upon a benevolent control agency to recognize these costs without an effort on their part to effect economies. C.J.B.

MISCELLANEOUS

235. Studies Comparing the Sanitizing Efficiencies of Hypochlorites and Quaternary Ammonium Compounds.

C. K. Johns, Dominion Dept. of Agr., Ottawa, Canada. Jour. Res., *25* F., 1: 76–91. 1947.

The germicidal speed of four commercial quaternary ammonium compounds has been compared with that of two hypochlorites against *Staphylococcus aureus, Bacillus panis* (vegetative cells and spores), *Micrococcus candidus,* cheese starter organisms, *Escherichia coli,* and *Pseudomonas aeruginosa,* using the glass slide technique. This technique, originally devised for comparing chlorine sterilizing agents, consists of immersing a sterile glass slide in a suspension of the organisms in diluted skim milk, allowing to drain, immersing in the sanitizing agent, rinsing in tap water, and finally transferring to a sterile petri plate, the plate immediately being poured with agar. A treatment which kills a calculated 99.9% of the test organisms is taken as standard. In addition to compounds tested, the effects of time, temperature, concentration, and reaction also were studied.

The quaternary compounds generally were more effective against the Gram-positive species, whereas the hypochlorites generally proved more effective against the Gram-negative species. Cheese starter organisms were an exception; they were killed faster by the hypochlorites. Three of the four quaternary compounds were comparable in efficiency, while the fourth definitely was slower. The hypochlorites responded much more readily to favorable adjustments in pH and temperature than did the quaternary compounds. Some bacteriostatic effect was observed with higher concentrations of all quaternary compounds. O.R.I.

236. The Mechanical Aspect of Can Washing.

A. F. H. Briscoe, U. D. Engineering Co., Ltd., London, England. Dairy Indus., *12,* 2: 127. Feb., 1947.

The author presents a very practical discussion of the mechanical aspects of can washing, using both rotary and straightaway machines. Indicating thermometers for washing and hot air drying stages as well as pressure gauges for all pumps are recommended equipment. Thermostatic control of washing temperatures also is desirable. Various types of construction are discussed and evaluated in terms of the comparative efficiency of can washing. D.V.J.

237. Rodent Prevention about Dairy Plants.

E. M. Searls, Sealtest, Inc., N. Y. C. Milk Dealer, *36,* 7: 45, 108–112. April, 1947.

Means of preventing rodents in dairy plants are: (1) Rodent proof the plant. (2) Select a bait the rodent wants. (3) Trap systematically—get an adequate number of traps and bait them all; set all of the traps the same evening; pick up all the traps the next morning, removing the bait and storing them away during the day; continue fresh baiting of traps, setting them each evening and picking them up the next morning until no rodents are caught for two successive nights; then pick up the traps and store them for 2 weeks, unless rodents are seen in the meantime; resume the procedure at the end of each 2 weeks regardless of whether rodents are seen. (4) Do not permit traps to remain in place about the plant during the day. (5) Do not use poisons. C.J.B.

238. Problems in the Design and Layout of Dairy Plants. R. T. SOUTHMAYD, Ramsey Laboratories, Cleveland, Ohio. Milk Dealer, *36,* 6: 136–142. March, 1947.

The relation of plot size, plant efficiency, room arrangement, mechanical equipment, and refrigeration to the layout and design of dairy plants is discussed. C.J.B.

239. Calculation of Heat Recovery with Continuous Blowoff Systems Utilizing Steam Regeneration. M. C. MCKEOWN, Cochrane Corp., Philadelphia, Pa. Milk Dealer, *36,* 6: 50–52. March, 1947.

The primary purpose of a continuous blowoff system is to recover heat which ordinarily would be lost when boilers are blown directly to the sewer. In a system employing the "flash" principle, heat is recovered by the utilization of steam flashed from the blowoff and by directing the hot waste water remaining after flash through a heat exchanger. Examples of how to determine the heat recovery and savings are given. C.J.B.

240. New Sweetening Agents. P. E. VERKADE, N. V. Polak & Schwarz's Essencefabrieken, Zaandam, Holland. Ice Cream Trade Jour., *43,* 3: 54. March, 1947.

Shortage of sugar in European countries during the war resulted in the development of several new materials which could be used as substitutes. One of these new compounds, known as 1-n-propoxy-2-amino-4-nitrobenzene, has a sweetening power 4100 times that of cane sugar. It now is being produced in Holland on a fairly large industrial scale and is used as a sweetening agent in many European countries. Several methods for production of this new class of sweetening agents have been worked out and are protected by patents or patent applications in many countries as well as in the United States.

Until recently the only synthetic sweetening agents used in practice were saccharine and dulcine, which are, respectively, 200–400 and 70–200 times as sweet as cane sugar. Before the present work was undertaken, the sweetest substance described in literature was perillaldehyde, an antialdoxime which seems to have been used as a sweetening agent in Japan and which is said to be about 2000 times as sweet as cane sugar. W.H.M.

241. Packaging. V. The Grease Resistance of Some Common Packaging Materials. C. G. Lavers, Natl. Research Laboratories, Ottawa, Canada. Jour. Res., *25* F., 1: 1–7. Jan., 1947.

The grease resistance of a wide variety of packaging materials was tested before and after creasing and aging. Kraft and sulphite were of little or no value, even after paraffin wax coating or impregnating. Vegetable parchment and greaseproof paper were superior to kraft. The grease resistance of glassine was 5 to 10 times greater than either greaseproof paper or vegetable parchment. Polyethylene, in turn, was considerably more resistant than glassine, and just slightly less resistant than all grades of "Cellophane" and cellulose acetate, cellulose nitrate, ethyl cellulose, Pliofilm, vinylite, and Saran. Coating glassine with paraffin increased grease resistance. Neither aging nor creasing appreciably affected the grease resistance of Cellophane or the thermoplastic films. O.R.I.—from author's abstract.

242. Supply and Demand in Dairy Industry. C. E. Lackner. Canad. Dairy and Ice Cream Jour., *26*, 4: 37. April, 1947.

In order to meet requirements for butter and cheese in Canada in 1947, increased production of milk will be necessary. The price differential is such that most of the milk now goes into market milk and concentrated milk products. Unfavorable climatic conditions, labor shortages on the farms, and the export of cows have decreased production. Herd improvement and higher efficiency are needed to get maximum production. H.P.

243. What Is Quality in Dairy Products? W. Vincent, Denver Station, U. S. Food and Drug Administration. Milk Dealer, *36*, 7: 144–149. April, 1947.

In addition to setting forth some of the sources of milk contamination on the farm and in the processing plant, the author points out that too much stress has been placed on some aspects of grade A milk production. He believes that ultimately there will be just two kinds of milk offered to dairy plants. One will be good, fit for any use, and the other will be good for nothing other than animal feed. C.J.B.

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Waugh, R. K.
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Weiser, H. H.

Yale, M. W.

JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal

Canadian Dairy and Ice Cream Journal
Canadian Journal of Public Health
Canadian Journal of Research
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World

Endocrinology

Food in Canada
Food Industries
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Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of the American Oil Chemists Society
Journal of American Veterinary Medical Association
Journal of Animal Science
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly
Molkeritidende

National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Nordisk Mejeri-Tidsskrift

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture
Southern Dairy Products Journal

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
Milk Industry Foundation

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BACTERIOLOGY

244. Fundamental Aspects of the Thermoduric Problem. W. A. CORDES, Sealtest, Inc., N. Y., N. Y. Milk Indus. Found. Assoc. Bul., *39*, 8: 195–208. Feb. 21, 1947.

Thermophilic bacteria are largely an in-plant problem, frequently resulting from old returned milk. On the other hand, the thermoduric problem is related to farm sanitation. Over a 5-month period the laboratory-pasteurized count of a plant receiving from 66 dairies was 28,360 per ml. Fifty machine-milking dairies averaged 31,000 pasteurized count and the 16 hand-milking dairies averaged 1,550 per ml. No hand-milking dairy had a high pasteurized milk count, which pointed to the milking machine as a cause of the trouble.

Milk stone from machine-washed cans was found to have a pasteurized plate count of zero. However, inflations and milk tubes showed a pasteurized count as high as 1,500,000 per ml. when rinsed with 50 ml. of sterile water; 0.1 g. of milk stone from a can in 5 ml. of sterile water gave 1,080,000 per ml. Pulsators and air lines also required attention. Many causes of poor cleaning and sterilizing results were found, but either dry or wet storage gave good results if used properly. For dry storage a final clean water rinse of at least 180° F. is required. A final 4-month period showed an average pasteurized count of 4,400, or less than one-sixth that of the initial 5 months. The value of a thermoduric program lies in the detection and elimination of dirty milking machines and insanitary practices of cleaning and handling. E.F.G.

CHEESE

245. Streamlined Cheese Making. W. FOUST, Belle Center Creamery and Cheese Co., Belle Center, Ohio. Natl. Butter and Cheese Jour., *38*, 5: 40. May, 1947.

A center-slung centrifuge is used to remove the whey from the curd at the time of dipping Cheddar cheese. The curd is left in the spinning centrifuge until it is ready for milling. Then it is cut into slabs, removed from the centrifuge, and run through the mill. The procedure saves space and labor; the vat can be used again for another batch of milk while the curd is spinning. An acidity of 0.25% can be developed safely in the whey before dipping; when this is done the curd can be removed from the centrifuge and milled after 10 min. and can be salted 20 min. later. The finished cheese is closer in texture, better in flavor, and lower in moisture than that produced by the conventional method. The method may lead to other mechanical improvements in handling curd. W.V.P.

CHEMISTRY

246. Monthly Variations in the Composition of Milk. O. R. OVERMAN, Univ. of Illinois, Urbana. Milk Indus. Found. Assoc. Bul., *39,* 8: 217–224. Feb. 21, 1947.

Results presented in Ill. Agr. Expt. Sta. Bul. 325 and Bul. 457 are discussed. Based on the analysis of 2,426 milk samples, monthly average values are given for Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey breeds for fat, protein, lactose, ash, total solids, solids-not-fat, and specific gravity. The percentage values for all components except lactose tend to be highest during the colder months. For lactose the tendency is toward higher values during the warmer months. E.F.G.

DISEASES

247. Raising Heifer Calves on Mastitis Milk. S. D. JOHNSON. Amer. Vet. Med. Assoc. Jour., *110,* 840: 179–182. March, 1947.

A preliminary report on the effects of raising heifers on mastitis milk tends to indicate that no pronounced detriment results. Of 12 heifers penned separately and pail-fed from infected udders, none showed *Streptococcus agalactae* at freshening, but 10 quarters were permanently infected with hemolytic staphylococci. Six heifers, penned together and pail-fed milk from infected udders, calved with only one positive reaction for *agalactae.* A large number of Guernsey calves were raised on nurse cows that were infected with mastitis. Examination of nine of the cows that had freshened and 15 that were near freshening showed no especially harmful results. T.M.L.

248. Vaccination against Brucellosis. C. M. HARING, J. TRAUM, AND W. E. MADERIOUS. Amer. Vet. Med. Assoc. Jour., *110,* 839: 103–107. Feb., 1947.

Although strain 19 of the *Brucella abortus* organism has been shown to be of definite value as an aid in the control of Bang's, the resistance to this strain by vaccination may be overcome by exposure to wholesale (massive) field infections. Vaccination with strain 19 has certain limitations in that it is a suspension of live organisms in physiologic saline solution and is a fragile product subject to rapid deterioration. The vaccination of heifers at ages approaching and subsequent to sexual maturity is relatively efficient. Vaccination of heifers at ages older than those commonly recommended gives satisfactory results.

It is recommended that negative adult cows be vaccinated to prevent sudden acute outbreaks of abortion. The practice of revaccination of adult animals, previously vaccinated as heifers, appears to be promising. There is considerable room for improvement in method and vaccine for Bang's.

T.M.L.

249. Vaccination Studies on Bovine Trichomoniasis. B. B. Morgan. Amer. Jour. Vet. Res., *7,* 26: 54–56. Jan., 1947.

To determine the efficiency of immunizing cows against *Trichomonas foetus* by intramuscular or intravenous injection of motile trichomonads, 30 virgin heifers, all about 12 months of age, were divided into three groups of ten each and injected with various concentrations of trichomonads. Thirty virgin heifers not injected with trichomonads served as controls.

Group A received 16 biweekly injections with concentrations increasing from 5 to 50 million organisms. Nine of these ten heifers calved normally. Trichomonads were isolated from all of the control group, but from only one of the experimental animals. In Group B the heifers were injected biweekly for 3 weeks with 5 cc. of a concentration of 50 million organisms per cc. Only three of these animals became pregnant and seven were heavily infected with trichomonads. The control group had four negative and six positive animals. Group C received only two injections over a 3-week period and eight of the ten heifers were heavily infected and none became pregnant.

Temporary immunity may result after a long series of injections, but the vaccination of heifers is ineffective for protecting breeding females and cannot be recommended as a practical procedure. T.M.L.

250. Clinical Use of Sulfamerazine in the Treatment of Hemorrhagic Septicemia and Pneumonia in Cattle. J. L. McAuliff, Amer. Vet. Med. Assoc. Jour., *110,* 842: 314–317. May, 1947.

An initial daily dose of 1.5 g. of sulfamerazine per lb. of body weight, administered in equal parts at 8-hr. intervals and followed by a dosage of 0.5 g. per lb. for 2 or 3 days thereafter, has proved very successful in relieving clinical symptoms of pneumonia and hemorrhagic septicemia. The drug had no apparent toxic effects. T.M.L.

251. Muscular Dystrophy (White Muscle Disease) in Young Calves. L. R. Vawter and E. Records. Amer. Vet. Med. Assoc. Jour., *110,* 840: 152–157. March, 1947.

A condition similar to "white muscle disease" or "stiff lamb disease", found extensively in young lambs under 1 month of age, has been observed in young beef calves. The disease was observed in calves of western beef herds wintered on poor-quality range hay. The largest number of cases occur in late winter and early spring; as soon as the cows are turned to pasture or given a supplement of good alfalfa hay, the incidence subsides almost immediately.

The condition usually occurs while the calves still are nursing (from 10 days to 2 months). Frequent bawling, inability to nurse, slightly stiff joints, and occasional small lesions on the tongue characterize the first observable symptoms. Skeletal and cardiac muscles are affected greatly, as determined by post mortems. Clinical observations at autopsy usually reveal skeletal muscle lesions or localized cardiac lesions. The malady occurs in two forms, the peracute and subacute. Fever seldom is recorded until paralysis or prostration occurs.

The symptoms and anatomical lesions observed in the calves closely resemble those found in spontaneous muscular dystrophy in young lambs and those induced in lambs, rats, rabbits, guinea pigs, and some birds by diets deficient in vitamin E. Although nutritional deficiency would seem a likely explanation of the disease, no positive conclusions have been drawn.

T.M.L.

FEEDS AND FEEDING

252. The Prophylactic Administration of Vitamins to Dairy Calves.

L. O. GILMORE, L. M. JONES, L. A. KANEGIS, AND M. H. ROEPKE. Amer. Vet. Med. Assoc. Jour., *110,* 843: 390–392. June, 1947.

Data from 159 unselected calves, born over a period of 3 years and representing 76 control and 83 experimental animals, indicate that the administration of vitamins (A, B, D) to young calves during their first month of life apparently is of no measurable benefit. The criteria for comparison of control and experimental groups were incidences of scours and pneumonia, gain in body weight, and apparent thriftiness. T.M.L.

ICE CREAM

253. Packaged Ice Cream.

H. B. GRANT. Ice Cream Field, *49,* 3: 10. March, 1947.

A recent survey indicates that one-third of the total volume of ice cream now goes into packages, whereas prior to the war it fluctuated somewhat below 20%. Of those polled, 82% listed the grocery store as the best outlet for their products, 64% reported satisfactory sales in drugstores, and 50% used frozen food stores as regular outlets. Only 7% listed department stores as outlets.

Seventy-five per cent indicated the need for redesigning their packages for better eye appeal. The tray-pack seemed to be the style package favored most, with 56% preferring it and 28% favoring the pail style container. Considerable divergence of opinion was expressed regarding the advisability of selling 6-cent, 7-cent, and 10-cent cones, cups, or novelties; 46% favored 10-cent items and 89% agreed that the "kids' nickel" still will buy something. W.C.C.

254. Production—Back on the Ice Cream Standard? E. L. Koepenick, Natl. Conference of Ice Cream Industries. Ice Cream Field, *49*, 2: 20. Feb., 1947.

Definitions and standards for ice cream, frozen custard, sherbet, and water ices are proposed. These are offered as suggestive of the type of definitions and standards which may be adopted for these and other foods. W.C.C.

255. Freezing Cream for the Ice Cream Manufacturer. G. M. Trout, Michigan Agricultural Expt. Sta. Food Freezing, *2*, 4: 203. Feb., 1947.

The two possible major defects to be overcome if frozen cream is to meet the demands of the ice cream manufacturer are "oiling off" upon defrosting and oxidized flavor. The only control of the "oiling off" factor is the use of 10–12% sugar in the cream to be frozen for subsequent use in ice cream mix. Avoidance of oxidized flavor is accomplished by the following: (1) Obtain cream only from high quality milk. (2) Handle milk and cream out of contact with copper. (3) Store only low-acid cream. (4) Store a cream testing 50% fat. (5) Pasteurize the cream at a high temperature to destroy pathogenic bacteria and reduce the numbers of non-pathogens, to inactivate the enzymes, particularly lipase, and to produce reducing substances (sulfhydryl compounds responsible for the cooked flavor) in the cream. A minimum pasteurization temperature of 165° F. for 15 min. is recommended for cream to be frozen for prolonged storage; a definite cooked taste must be developed. (6) Avoid air incorporation in the cream. (7) Package and store cream in non-absorbent, impregnated paper containers. (8) Sample the cream before freezing, code the containers, and taste the cream at various intervals of storage. L.M.D.

256. Bananas—North of the Border. P. R. Foote, The Standard Fruit Products Co., Cincinnati, Ohio. Ice Cream Field, *49*, 2: 40. Feb., 1947.

Bananas of the highest quality are obtained when the fruit is allowed to ripen in the sun. Processing properly ripened "Lady Finger" or Chinese variety bananas as puree in modern sanitary plants in the heart of Cuba results in a product that serves as a good source of flavor for ice cream. Since this product was introduced to the ice cream industry in the United States slightly over a year ago, thousands of loads have been distributed. Imports are stabilized on a year-around basis. W.C.C.

257. Effect of Freezing Storage on Strawberries, Blackberries, Raspberries, and Peaches. J. G. Woodroof and Ethyl Shelor, Georgia

Experiment Station and TVA, cooperating. Food Freezing, *2*, 4: 206. Feb., 1947.

Strawberries, blackberries, raspberries, and peaches were frozen and stored at −10°, −20 to 0°, 0 to 10°, and 10° F. for 1 year. Examinations were made after 3, 6, 9, and 12 months. Records were made of appearance, color, aroma, flavor, texture, leakage, and resistance to crushing.

Desirable appearance, color, aroma, texture, and flavor of strawberries were preserved much better at −10° F. than at higher temperatures or at fluctuating temperatures. Strawberries were in better condition after 12 months' storage at −10° F. than after 6 months at 10° F. All of the fruits deteriorated more rapidly at a temperature fluctuating from 0 to 10° F. than when held constant at 10° F.

Blackberries and raspberries stored at −10° F. were superior to those stored at higher temperatures, but the differences were not so great as those found with strawberries and peaches. All samples of frozen blackberries and raspberries developed a semi-cooked aroma and flavor; blackberries had a semi-cooked color. Peaches deteriorated more rapidly in flavor and color than did the other fruits at the four temperatures indicated. They also seemed to lose quality more rapidly at fluctuating temperatures than did the other fruits.

In these experiments sugar or sirup was not used in order that firmness could be determined with a tenderometer. Antioxidant was not used with the peaches in order that natural color retention could be observed at the various storage temperatures through the 12-month period of observation.

L.M.D.

258. The Effect of Various Pretreatments of Frozen Peaches on Quality. Lenore E. Sater, Mary E. Kirkpatrick, Kay E. Stein, Hazel B. Murray, and Dorothy G. Skinner, Bureau of Human Nutrition and Home Economics, Agr. Research Admin., U. S. Dept. of Agr. Food Freezing, *2*, 3: 128–131, 144. Jan., 1947.

The color, flavor, texture, ascorbic acid content, and enzyme activity of peaches frozen and stored under home conditions were studied to determine the effects of various treatments and packs. Treatments to prevent browning consisted of dipping for 1 min. in citric acid, Frulite, and sodium bisulfite and then packing in 40% sirup or a 3:1 ratio of fruit to dry sugar, using Golden Jubilee and Elberta varieties. September Elberta peaches were packed in 40 and in 60% sirup containing various amounts of ascorbic acid. Analyses for palatability, enzyme activity, and ascorbic acid content were made on the fresh and freshly-frozen peaches, and at the end of 4, 8, and 12 months of storage.

Dipping in a 0.75% sodium bisulfite solution before packing resulted in a very objectionable flavor and a bleached color. Although all of the other four treatments were satisfactory, peaches packaged in ascorbic acid-sucrose sirup and those dipped in Frulite before packaging were the most successful. Regardless of treatment, all the 40% sirup-packed peaches were superior to the corresponding dry sugar packs in all respects.

(Abstractor's note: At the present time the Food and Drug Administration does not permit the addition of Frulite (Thiourea) to commercial foods because its safety has not been demonstrated clearly.) L.M.D.

MILK

259. The Relationship between Atmospheric Temperature and the Frequency of Low-grade Milk Samples in Devon. H. H. MITCHELL. Dairy Indus., *12,* 4: 351–352. April, 1947.

A 3-year study involving the milk from about 8,000 producers demonstrated conclusively that a close relationship exists between atmospheric temperature and the quality of the milk received. When the prevailing temperatures were 45° F. or less, the amount of Grade C milk received was below 5%. During summer months when mean temperatures were between 60 and 65° F., the amount of inferior grade milk approached 20 to 30%. A graphic chart covering the 3-year study is presented. D.V.J.

260. Planning an Efficient Receiving Room. V. SCHWARZKOPF, Lathrop-Paulson Co., Chicago, Ill. Milk Dealer, *36,* 5: 48. Feb., 1947.

Some of the important considerations in planning the receiving room and in selecting the proper equipment for highest efficiency under a given set of conditions are: (1) Volume of milk to be received per hour, (2) number and size of cans to be received per hour, (3) number of producers and their average daily delivery of milk, (4) accessibility of the room for getting cans in and out, (5) relative elevation of driveway and receiving room floor, and (6) relative location of receiving room to driveway or street. Arrangement and equipment, the conveyor system, dumping and weighing of milk, the can washer, and ventilation are discussed. C.J.B.

261. Pasteurization of Milk. Current Comment. Amer. Med. Assoc. Jour., *133,* 4: 249. Jan. 25, 1947.

This editorial encourages compulsory pasteurization of milk and decries irresponsible persons who do not recognize the advantages of the pasteurized product. D.P.G.

262. Quality Control in Dairy Plants. E. S. GUTHRIE, Dept. of Dairy Indus., Cornell Univ., Ithaca, N. Y. Milk Dealer, *36,* 5: 144–149. Feb., 1947.

The effects of dairy utensils, feeds, lipase, copper, and sunlight on the quality of milk are discussed. The author recommends the use of hot water for sterilizing utensils, preventing feed flavors by feeding silage and other highly flavored feeds immediately after milking, preventing the development of a rancid flavor by heat treating the milk, and preventing oxidized flavors by eliminating copper in the equipment that comes in contact with milk and avoiding the exposure of the milk to light. Improving the keeping quality of milk by deaeration and preventing the development of oxidized flavors by destroying the vitamin C also are discussed. C.J.B.

263. Relations of Metals to Milk. F. R. Pattison. Dairy Indus., *12,* 4: 329. April, 1947.

The corrosion of the various types of metals used in dairy equipment and utensils is discussed. The effect of protective films set up on the surface of nickel, by hot milk, is pointed out. This effect is specific for hot milk on nickel and does not hold for other fluids or other metals. The influences of temperature, oxygen content, rate of flow and acidity of milk are discussed, as well as the possibility of galvanic effects with certain metals. Aluminum is highly resistant to corrosion by milk, but its inferior mechanical properties and low resistance to brine and alkali limit its use in dairy equipment. High nickel alloys are emphasized; their resistance to corrosion, their strength, thermal conductivity, and adaptability to fabrication make them very suitable for dairy equipment. D.V.J.

264. Streamlining Plant Operations, Distribution. R. W. Blackburn, Safeway Stores, Inc., Los Angeles, Calif. Milk Dealer, *36,* 5: 152–155. Feb., 1947.

Methods used by Safeway in streamlining its milk operations are discussed. Storage tanks are used in place of milk cans on the farm and for transporting the milk to the plant. This streamlining is more economical; tank hauling resulted in a saving of 0.054 cents per gallon or 6.27 cents per 100 lb. of milk. Time and labor at the producing dairy are saved; approximately 15 min. a day is required to wash and sterilize the storage tank as against 3 hr. a day for cans. The weighing (measuring) and sampling of the milk are more accurate and the quality of the milk is improved. The advantages of using paper containers and of selling milk through stores are discussed. C.J.B.

265. Cost Reduction in the Bottled Milk Industry. C. H. Zamzow, Jr., George Fry and Associates, Chicago and New York. Milk Dealer, *36,* 5: 45. Feb., 1947.

One of the most urgent problems confronting milk dealers today is to increase productivity while reducing costs. The part that complete production engineering programs can play in solving these problems is discussed under the following headings: (1) What is production engineering? (2) Results to be expected from a program. (3) Production engineering department. (4) Developing the program. (5) Training the production engineering department personnel. (6) Gathering basic data upon which to base the program. (7) Familiarize management and labor with production engineering. (8) Work simplification and methods analysis. (9) Job evaluation. (10) Establishment of production standards. (11) The establishment of production schedules. (12) Installation of a wage incentive plan. (13) Cost control, maintenance, and follow-up. In the final analysis, the goal of every business is a better product manufactured at the lowest possible cost in order that competition in the consumers' market may be met successfully. A production engineering program properly applied and maintained is the direct road to that goal. C.J.B.

266. Experiments in Changing Food Habits. MARIAN RADKE AND DAYNA KLISURICH, Child Welfare Research Sta., State Univ. of Iowa, Iowa City. Amer. Dietet. Assoc. Jour., *23,* 5: 403. May, 1947.

The group decision method was found superior to the lecture method in increasing consumption of bottled milk and evaporated milk in a series of experiments on changing food habits.

In the group decision method, groups of six to nine women met together and a group leader gave information about the nutritive value of milk and ways to increase its consumption by the family. Discussion of the subject and a decision by the group to increase the milk consumption of their families followed. Four weeks following the meeting, 50% of the women had increased their families' consumption of bottled milk and 50% had increased their families' consumption of evaporated milk as compared to corresponding percentages of 14 and 33 in the groups to whom a lecture on the nutritive value of milk was given.

Similarly, when the group decision method was compared with individual instruction in determining whether mothers followed recommendations regarding the use of cod-liver oil and orange juice in feeding their newborn infants, the group decision method was found superior in producing the desired response. M.B.

267. Chocolate Milk. R. A. SIMONET, Robert A. Johnston Co., Milwaukee, Wis. Milk Dealer, *36,* 5: 150, 151. Feb., 1947.

The manufacture of chocolate milk is discussed. Some of the causes of unsatisfactory results are: (1) too much milk; (2) too little powder or sirup; (3) too low holding temperature at pasteurizer; (4) insufficient cooling at surface cooler; (5) vat cooling; (6) lack of proper agitation while batch is hot at pasteurizer; (7) excessive agitation of finished (cooled) chocolate drink; (8) breakdown at bottler or washer, allowing last few gallons in pasteurizer to stand without agitation and paddles not long enough for proper agitation; (9) running hot batch from pasteurizer over surface cooler more than once; (10) allowing finished bottles of chocolate drink to remain too long at room temperature; (11) small hand-operated batch when 10 or 20 gallons of chocolate milk are held in receptacle above small surface cooler without agitation; (12) lack of proper increase of holding temperature for excessive pipes, elbows, and homogenizers; (13) improper storage of flavoring; (14) lack of rotation of chocolate flavoring in stock room; and (15) pumping finished cold chocolate milk without additional heat to compensate for agitation in the cold pump.

Jelling usually is caused by too little milk, too much flavoring, too much heat at pasteurizer above standard set, or insufficient agitation at pasteurizer. Jell once formed in the bottle can be broken by agitating the bottle or shaking the pile of milk crates in storage; jell does not return. Settling cannot be corrected in the finished bottle. C.J.B.

SANITATION AND CLEANING

268. Cleansing of Dairy Utensils. I. The Bacterial Content of Utensils Sterilized by Approved Methods.

S. B. THOMAS AND ELEANOR JONES-EVANS. Dairy Indus., *12*, 4: 347. April, 1947.

The efficiency of three types of farm sterilizers was investigated. Utensils were checked for sterility by rinse and swab techniques, using yeastrel milk agar for colony counts.

All sterilizing units were found to be satisfactory; the steam produced by each apparently provided efficient sterilization. Thermoduric counts were 3,500 per utensil for sterilized equipment and 133,000 for washed (warm water) utensils. The importance of removing milky residues from utensils before sterilizing is pointed out. Frequent or periodic servicing of sterilizing units is recommended. D.V.J.

269. Bacteriological Aspects of Can Washing.

C. A. SCARLETT. Dairy Indus., *12*, 5: 430–434. May, 1947.

Cleaning and sanitizing of milk cans are discussed under the headings of washing, sterilizing, and drying. Both mechanical and hand cleaning procedures are considered. The removal of all organic matter is emphasized and the relative merits of chemical and steam sterilization are discussed. Steam sterilization is more satisfactory because of its penetrating power and drying action. If the bacteria count per ml. of capacity of the can (rinse count) does not exceed that of the milk, there is little danger of serious deterioration of milk placed in the can. Clean dry cans will remain in a satisfactory condition for 2 days after cleaning and sterilizing. D.V.J.

270. **Washing and Sterilizing of Milk Bottles.** W. A. Josephson, Sheffield Farms Co., Inc., N. Y. C. Milk Indus. Found. Assoc. Bul., *39,* 8: 209–216. Feb. 21, 1947.

Investigations revealed that all sizes of bottles consistently met operating standards for bacterial counts when they emerged from the final caustic soak, providing proper caustic strengths and temperatures were maintained. However, bottles have been recontaminated and high bottle counts have resulted from contaminated rinse water, improper chlorination, improper type and adjustment of chlorine jets, and faulty sample collection technique. Drawings illustrate how changes in four types of bottle washers resulted in improvements. Detailed directions are given for taking washed bottle samples. E.F.G.

271. **A New Approach to the Bottle Washing Problem.** J. C. L. Resuggan and J. G. Davis. Dairy Indus., *12,* 5: 443–454. May, 1947.

A new non-foaming tetra-alkyl ammonium compound, di-n-octyldimethylammoniumbromide, has been developed in England. This cationic wetting agent has strong bactericidal powers. Comparisons were made between it and another common cationic agent producing foam and between it and well-known alkaline powder detergents. The greater efficiency of the cleaner containing the new cationic agent suggests that the use of non-foaming quaternary ammonium compounds may be an advance in the cleaning of milk bottles. Work is continuing. D.V.J.

MISCELLANEOUS

272. **Principles of Refrigeration Applied to Storage Space.** P. B. Christensen, Merchants Refrigerating Co. Food Freezing, *2,* 3: 135–137. Jan., 1947.

Explanations of relative humidity, moisture vapor pressure, and dew point and their importance in connection with refrigerated spaces are given. Charts illustrate the drying power of air at low temperatures with fixed humidity and varying temperatures or at varying humidities and temperatures. Relationships of these factors to insulation are emphasized. Vapor seals must be used on both sides of cooler insulation where outside temperatures go below the inside cooler temperature; in the case of freezers, only a warm side vapor seal is needed. L.M.D.

273. **Industrial Wastes—Dairy Industry.** H. A. Trebler and H. G. Harding, Natl. Dairy Research Lab., Inc., Baltimore, Md. Indus. Engin. Chem., Indus. Ed., *39,* 5: 608–613. 1947.

Dairy products are highly concentrated and perishable, and their wastes have a high pollution value. The trend is toward large manufacturing plants equipped to utilize all by-products. Although the large plants help to utilize waste from near-by small plants, waste disposal still is a serious problem in many scattered small plants.

The dairy industry has several kinds of waste which often may be treated separately. General domestic waste from lavatories, locker rooms, and laundries should go to septic tanks or city sewers. Boiler blow-off, which is alkaline and hot, is beneficial to septic and digester tanks and may be discharged into them. Backwash and regeneration rinse waters and cooling waters generally should be segregated and sent to storm sewers or into streams. Care must be taken in the disposition of spoiled products. Sometimes the most practical method is to truck them out for dumping in some isolated spot. By-product wastes, such as whey, buttermilk, and excess skim milk, are the worst offenders in stream pollution. These wastes should not be dumped into clean streams or small city sewers; they are not handled economically in either city or private industrial sewage disposal plants. Improved by-product utilizations should gradually decrease the waste from this source.

Waste from drips and leaks should not occur, but if present they sometimes may be recovered for animal food. Wash waters may require some kind of treatment before they are discharged into small streams. A reliable method for flow measurement and sampling should be provided; an automatic sampler is described. An aerated flow equalizing tank, which will distribute the load and provide some biochemical oxidation, is needed when dairy waste is to be discharged into a stream or into a small city disposal plant. The necessary treatment for waste should be given, if possible, in combination with existing or future city waste disposal plants. If these means are not available, the trend is toward treatment or recirculating trickle filters. Efficient and economical control, maintenance, and supervision of industrial waste disposal plants are needed and may have to be met on an industry-wide basis in the future. B.H.W.

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ABSTRACTS OF LITERATURE

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Prussian Dairy Research Institute, Kiel, Germany

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The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEW

274. **Animal Nutrition.** Second Edition. Leonard A. Maynard. 494 pages. $5.00. McGraw-Hill Book Co., New York. 1947.

The second edition of this well-established and excellent text follows closely the pattern of the first, which was published in 1937. The major purpose of the book, according to the author, is to teach how the facts of nutrition are being learned experimentally and how they find application in practical livestock feeding.

Like the original text, the new edition contains 16 chapters outlining the principles of nutrition and their applications in feeding practice. These deal, respectively, with the expanding field of nutrition, the animal body and food, physiochemical bases of life process, metabolism of the carbohydrates, lipids, proteins and the inorganic elements, vitamins, feeding experiments and determination of digestibility, nutritional balances, measures of food energy, maintenance, growth, reproduction, lactation, and work. The book also contains an entirely new feature, namely, a list of visual aids.

Extensive revisions and additions have been made to bring the subject matter up to date through March, 1946. Because of wartime conditions, most of the literature citations refer to work done in the United States. Much new information is presented about the proteins, minerals, and vitamins. The discussions of cobalt, manganese, and others of the so-called trace mineral elements in animal nutrition have been greatly expanded. Newly discovered rôles of carbohydrates and fats in animal nutrition are discussed. New facts are presented concerning the nutrient, mineral, and vitamin requirements of animals and about the comparative value of various feedstuffs for supplying those needs. Modifications of many ideas previously held are presented as based on recent experimental evidence.

The book is very well written, well illustrated, and well arranged. The author has done an excellent job of eliminating extraneous material. In spite of the fact that nutrition is to a large extent a chemical process, the subject matter is presented so clearly as to be readily understood even by those with little or no training in this field. Throughout the text, the author presents facts on the problems confronting the field of nutrition, on special applications to human nutrition, and on pioneer contributions of many individuals. These all help to add interest and value to the book. Cross references and the references listed at the end of each chapter help to increase the usefulness of the text as a source of information. T.W.G.

BACTERIOLOGY

275. Studies on Coliform Organisms in Dairy Products. L. BUCHBINDER AND EDYTHE ALFF, Bureau of Laboratories, Dept. of Health, New York City. Jour. Milk and Food Technol., *10*, 3: 137–148. May–June, 1947.

A survey of the coliform incidence in pasteurized milk in New York City was made for the purpose of establishing coliform standards. The question of heat resistance of coliforms in milk is of little significance.

The following conclusions were reached: (a) the reports in the literature on heat resistance of coliforms frequently are fragmentary and inconclusive; (b) many of the reports are based on laboratory results and not in a milk plant itself; and (c) differences in techniques employed in the study, such as partially submerged tubes, an unspecified number of organisms used, and, in many instances, a reporting of greater numbers of heat resistant organisms than normally would be found in raw milk, give a wide variation in the results.

The authors attempted to carry out three approaches to the problem. First, they determined the frequency with which coliforms are present in milk just leaving the holder in a pasteurizing plant. In many instances as few as five coliforms per liter of milk were found. Repeated tests over a period of several months showed that coliforms rarely escape destruction in the holder, being detected in only one of some 400 liters of milk sampled. Secondly, in only about 2% of the samples studied in a survey of the raw milk supply of New York City over a period of 1 year did the coliform count exceed 100,000 per ml. Thirdly, the authors found that when raw milk was pasteurized by the partial submergence technique, 7.5% of 482 samples showed coliforms. The presence of survivors was unrelated to the initial numbers present in the raw milk. The same method was used to test 18 pure cultures of the survivors in a total of 21 trials. The cultures each were tested in four decimal dilutions falling within the range of coliforms found in natural raw milk. Of the 84 tubes, 46 (55%) were positive. The survivors seemed to be distributed by chance among the several dilutions. Ten of the same strains were investigated in some 36 tests, using the complete-submergence, sealed-tube method, with the initial numbers ranging from 3 million to 396 million per ml., and thus much greater than would be expected in normal raw milk. No coliforms were recovered after 30 minutes. Shorter time intervals showed 8 of 25 cultures survived after 0 minute, 3 of 25 after 5 minutes, but none after 10 or 15 minutes. These results indicate that coliforms do not survive the time and temperature of laboratory pasteurization when the proper technique is used.

H.H.W.

276. **What Is Meant by Coliform Bacteria.** R. N. Doetsch, Dept. of Bact., Univ. of Maryland, College Park. Milk Plant Monthly, *36,* 1: 89–91. 1947.

Coliform organisms are defined as "all aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacteria which ferment lactose with the production of gas". They are members of a large group of bacteria which have many characteristics in common. The presence of coliform bacteria in milk indicates improper pasteurization or post-pasteurization contamination. Dairy bacteriologists use all the coliform organisms as indicators of faulty procedure. Several methods are described for detecting coliform bacteria in dairy products. G.M.T.

277. **Coliform Bacterial Contamination of Milk and Milk Products. Practical Problems and Their Solution.** R. P. Myers, Natl. Dairy Products Corp., Research Laboratories, Baltimore, Md. Milk Plant Monthly, *36,* 2: 30–33. 1947.

The coliform test, widely used in the dairy industry by plant operators, is particularly helpful in checking the efficiency of cleaning and sterilizing procedures. Experience in plant pasteurization of milk has demonstrated rather conclusively that coliform bacteria do not survive proper pasteurization, either by the holding or short-time method. Thus, if coliform bacteria are found, post-pasteurization contamination of the milk has resulted. The sources of coliform contamination may be as follows: (a) improperly sterilized equipment, (b) contamination of equipment when assembled, (c) condensate dripping from pipes, etc., (d) handling equipment which contacts dairy products, (e) air contamination, (f) contamination of pasteurized milk with improperly pasteurized milk, (g) vacuum lines at the bottle fillers, (h) contaminated colors, flavors, and fruits, particularly in ice cream, (i) rubber gaskets and packing glands, (j) dead-end tees, and (k) seepage through plug caps. Most effective sterilization is brought about by combination of both hot water and chemicals. Coliform bacteria grow at relatively low temperatures. Thus, the tests should be made on the fresh product rather than on a sample stored for some time in the refrigerator. The coliform tests when intelligently used in the plant aid in locating the points which need more attention in sanitation. G.M.T.

278. **An Occurrence of Udder Infections Caused by a Thermoduric Streptococcus.** J. S. Bryan, Walker-Gordon Laboratory Co., Plainsboro, N. J. Milk Plant Monthly, *36,* 3: 32–33, 92. 1947.

Many thermoduric bacteria come from the udder of the cow. Cases are cited in which udders were infected by thermoduric bacteria on inadequately washed milking machines, resulting in high thermoduric counts in the milk.

Recommendations in the control of thermoduric streptococci in a herd or milk supply are: (a) constant vigilance and checking to discover infected cows and keeping milk of such cows out of the supply, (b) careful cleaning of milking machines to prevent spreading of organisms to other cows, and (c) careful cleaning and sterilizing of the pasteurizing and bottling equipment. G.M.T.

BUTTER

279. The Washing of Butter and Its Effect on Curd Content and Quality.

E. G. Pont. Austral. Council Sci. & Indus. Res. Jour., *19,* 4: 432. 1946.

The presence of increased curd in the range 1.1% for washed butter and 1.52% for unwashed butter is not a determining factor in keeping quality. Unwashed butter involves adverse factors not determined in this investigation. Out of 74 churnings of butter, only two unwashed samples graded higher by one point after 1 week's storage. As the storage period advanced, the washed butter scored higher in most instances. After 3 months' storage, washed butter lost 1.23 points in grade while unwashed butter lost 1.71 points. There was no relationship for the inferior keeping quality of the unwashed butters and curd content, initial quality, and bacteria content. H.P.

280. Increased Costs of Operating Today's Butter Business.

P. Potter, Paul Potter & Associates, Chicago, Ill. Natl. Butter and Cheese Jour., *38,* 7: 46. July, 1947.

Responses to questionnaires representing 79 butter manufacturing plants show increased costs. These costs increased from 1940 to 1945 as follows: manufacturing costs, 51%; print room, 41%; overhead, 30%; and total costs, 41% (not including selling expense). Estimated increases in procurement cost averaged about 42%. The total unit cost of manufacturing, printing, and overhead increased about 1.4 cents. Increased costs per unit were due to reduced volume as well as to higher costs of labor and materials. W.V.P.

281. Dasher for Churns.

H. R. Marshall. U. S. Patent 2,421,798, June 10, 1947 (3 claims). U. S. Patent Office Off. Gaz., *599,* 2: 233. 1947.

The surfaces of a blade for a rotary churn dasher are designed to improve the churning operation. R.W.

CHEESE

282. The Control of the Early Gas Defect in Cheese Caused by the Coli-Aerogenes Bacteria.

TH. E. GALESLOOT, Netherlands Milk and Dairy Jour., *1*, 1: 33–42. 1947.

The early gas defect in cheese could be controlled with either KNO_3 or KNO_2. These compounds must be added in an amount sufficient to delay gas production from lactose until gas production can be checked sufficiently by the development of acid by the lactic acid organisms. $KClO_3$ also was suitable for controlling the early gas defect in cheese; a sufficient amount must be added so that the gas-forming organisms can reduce this compound until the lactic acid bacteria have increased sufficiently to inhibit the gassy fermentation. The objectionable flavor caused by the coli-aerogenes bacteria was not as pronounced when $KClO_3$ was used as when KNO_2 or KNO_3 was used. The author favors the use of $KClO_3$. F.J.B.

283. Cottage Cheese Manufacture. Part 1. Its Problems and Possibilities.

W. I. TRETSVEN, Dairy Advisory Service, Chicago, Ill. Milk Plant Monthly, *36*, 5: 24–27, 32. 1947.

Production of cottage cheese is increasing rapidly since the quality has improved. This item is economical, is generally liked, and is profitable for the dairy manufacturer. Methods of manufacture are presented in considerable detail. Difficulties sometimes encountered are discussed and remedies suggested. The writer believes that the production of cottage cheese is plagued with superstitions and undesirable cure-alls, which may be wasteful, illegal, or questionable. With general improvement in quality, increased consumption may be expected. G.M.T.

284. Cottage Cheese Manufacture. Part 2. Creaming and Packaging of Cottage Cheese.

W. I. TRETSVEN, Dairy Advisory Service, Chicago, Ill. Milk Plant Monthly, *36*, 6: 26–29, 32. 1947.

Since cottage cheese is mostly retailed as a packaged product, several problems of creaming and packaging cottage cheese are present. Creaming mixtures vary markedly in the percentage of fat. Details are given relative to the creaming of cottage cheese. Hand packaging procedures are criticized because of lack of sanitation, lack of uniformity, high labor costs, obsolescence, and lack of dependability of labor. Precautions must be taken to reduce contamination of cottage cheese. The yeast and mold content is a fair index of the sanitary condition of the product. A phosphatase and sediment test are essential to determine the effectiveness of pasteurization and general cleanliness. G.M.T.

285. Manufacture of Cheese. W. Falhndrich. U. S. Patent 2,423,300, July 1, 1947 (2 claims). U. S. Patent Office Off. Gaz., *600*, 1: 121. 1947.

A perforated tube is placed in a position approximating the axis of cheese at the time of manufacture, to facilitate drainage of the whey from the center, thereby improving the texture of the cured cheese. R.W.

CHEMISTRY

286. The Mechanism of the Oxidant Effects of Commercial Salt and Water in Butterfat. G. L. Hills and J. Conochie. Austral. Council Sci. & Iudus. Res. Jour., *19:* 414–429. 1946.

The catalysis of the oxidation of the fat in cold storage butter by salt and acid present in the water phase was studied at 40° C. using hydrogenated peanut oil to give solidity to the fat and to maintain the necessary fine moisture distribution. Dispersed water alone in butterfat accelerated oxidation. Catalysis of fat oxidation by commercial salt in acid solution was found to be due to the combined effects of halide and hydrogen ions. The effect is a direct one between acid salt-solution and fat; the presence of other butter constituents is not necessary. The mechanism suggested and supported by experimental evidence involves the formation by reaction between fat-hydroperoxide and hydrogen and chloride ions of chlorine, which then activates further oxidation. The previously reported oxidant effects of traces of magnesium chloride in commercial salt, when the salt is dispersed in the dry state in fats and oils, can be explained similarly. In butterfat, glyceryl monostreate added at the rate of 0.1 to 1.0% was found effective in stopping the reaction at the interface between the fat and salt solution. H.P.

287. Note on the Effect of Phosphatide on the Ferric Thiocyanate Method of Estimating Peroxide in Fats. G. L. Hills and R. Wilkinson. Austral. Council Sci. & Indus. Res. Jour., *19*, 4: 430. 1946.

Milk phosphatide at a concentration of 0.05% was found to interfere with the ferric thiocyanate test for peroxide groups in fats. This does not cause difficulty in the application of the test to dairy products other than dried buttermilk when benzene-methanol is used as the solvent. Acetone will not dissolve phosphatide to cause interference. H.P.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

288. Concentrated Milks. B. H. Webb, Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Dept. of Agr. Milk Plant Monthly, *36*, 1: 44–46, 48, 50, 52. 1947.

Described briefly are the history of the concentrated milk industry, the composition of various concentrated milks, their methods of manufacture, their nutritive values, and the economic factors necessary for the successful establishment of a condensery, namely, location in the dairy community, location in a small town, good transportation facilities, abundant water supply and good drainage, an available market for the products, and sufficiently large capital. G.M.T.

289. **From Pan to Package. The Importance of Cooling Process in the Manufacture of Sweetened Condensed Milk.** O. F. HUNZIKER. Milk Plant Monthly, *36,* 6: 74–76, 78–79. June, 1947; Natl. Butter and Cheese Jour., *38,* 7: 40. July, 1947.

The cooling process is one of the most important steps in the entire manufacture of sweetened condensed milk, for the type and number of sugar crystals will be determined largely by the treatment of the product during the cooling period. Sweetened condensed milk with very smooth texture and free from sugar crystal deposits is accomplished through mass crystallization. Seeding is indispensable in securing mass crystallization, the logical seed material being powdered lactose. The pulverized lactose may be added at the rate of 4 to 6 ounces of seed lactose per 1,000 lb. of original fluid milk. Several methods of cooling may be employed, each of which has advantages and disadvantages. G.M.T.

290. **The Future of Dry Milk.** S. O'DEA, Production and Marketing Administration, U. S. Dept. of Agr. Milk Plant Monthly, *36,* 1: 30–31, 64. 1947.

While the utilization of dry milk and nonfat dry milk solids has been high during the war years and many new uses for them have been found, the long-run post-war demand for milk powder is uncertain. Development of markets for nonfat dry milk solids is more imperative than for any other manufactured dairy product. G.M.T.

291. **Desiccating Method and Apparatus.** G. TOOBY. (Assigned to Richard C. Lilly.) U. S. Patent 2,422,706, June 24, 1947 (10 claims). U. S. Patent Office Off. Gaz., *599,* 4: 593. 1947.

A spray drier designed for milk is described. The milk is introduced into a stream of hot air traveling toward the periphery of a conical-shaped chamber. The denser dried milk particles are removed from the bottom of the cone-shaped chamber, while the moisture-laden air leaves through a duct at the top to enter a cyclone collector. R.W.

DISEASES

292. The Prevention, Control and Treatment of Mastitis. C. S. Bryan, Dept. of Surgery and Medicine, Michigan State College, East Lansing. Milk Plant Monthly, *36*, 2: 97–98. 1947.

Mastitis is a very general term which refers to any inflammation of the udder regardless of the cause and severity of the condition. The most common cause of chronic infection is *Streptococcus agalactiae*. Available scientific data, substantiated by field trials, for the successful prevention and control of bovine mastitis involve good sanitary milking procedure, good sanitary herd management, early and accurate diagnosis of specific trouble, elimination by slaughter or complete segregation of cows with badly damaged udders, and early and proper treatment. G.M.T.

ICE CREAM

293. Technical Literature of Ice Cream for 1946. A. Leighton, Bureau of Dairy Industry, U. S. Dept. of Agr. Ice Cream Rev., *30*, 11: 45. June, 1947.

This is the ninth annual review of ice cream literature by the author. In the current review, a total of 249 articles are cited, covering all phases of ice cream manufacture. The bibliography, classified according to subject matter, will afford a particularly valuable reference to ice cream manufacturers and technical or scientific workers. W.J.C.

294. Carboxymethylcellulose in Ice Cream. M. Landers. (Assigned to Lanco Products Corp.) U. S. Patent 2,423,600, July 8, 1947 (10 claims). U. S. Patent Office Off. Gaz., *600*, 2: 253. 1947.

Inclusion of a water-soluble salt of carboxymethylcellulose in an ice cream mix improves the texture of the frozen product without adversely affecting other characteristics. A mixture to be added to the mix consists of 30 parts sodium carboxymethylcellulose having a viscosity of 1,500 centipoises in 1% aqueous solution, 15 parts glycerol α-stearate γ-benzoate and 55 parts cane sugar. One part of this mixture is added to 200 parts of ice cream mix containing 10% fat. Less is required for mixes containing greater percentages of fat. All the claims include a mixed glycerol diester of a higher fatty acid and an aromatic carboxylic acid in addition to the water-soluble salt of carboxymethylcellulose. E.O.W.

295. Shrinkage in Ice Cream. C. D. Dahle, D. J. Hankinson, and J. A. Meiser, Jr. Dairy Dept., Pennsylvania State College. Milk Plant Monthly, *36*, 1: 32–33, 36–38. 1947.

Shrinkage in ice cream is the most important unsolved problem in the ice cream industry today. Use of paper containers, continuous freezing, dry ice, high cabinet temperature, time of the year, and presence of free fatty acids, egg yolk, certain polyhydric alcohol esters, and certain sugars are among the contributing factors. A definite solution to the problem has not been found, and all the conditions thought to cause shrinkage may be present yet shrinkage may or may not occur. G.M.T.

296. Ice Cream Cabinet. R. J. SHARP. U. S. Patent 2,423,207, July 1, 1947 (1 claim). U. S. Patent Office Off. Gaz., *600,* 1: 98. 1947.

The sleeve of the cabinet is inclined toward the front of the cabinet to facilitate dipping of the ice cream. A pedal, pressed by the foot, causes the lid to slide upward, permitting access to the ice cream, the lid returning to the closed position on release of the pedal. R.W.

297. Customer Surveys as a Means of Checking up on Company Service. ANONYMOUS. Ice Cream Rev., *30,* 11: 46. June, 1947.

The results of a questionnaire submitted to customers at 13 Franklin Creamery Co. ice cream stores in Toledo and Cleveland, for the purpose of checking up on store operations, are summarized. A total of 2,095 replies were received, of which 76% contained comments of a favorable nature, whereas the remainder registered complaints on some phase of Franklin store operations. Specific things most frequently commented on favorably were: good quality ice cream, creamy texture of ice cream, good service, clean, attractive stores, good variety of flavors, availability of special molds and rolls, and convenient location of the store. Of the criticisms offered, poor service ranked first in number of replies, followed in order by unsatisfactory quality of ice cream, lack of table service, insufficient help, not open long enough, tables not cleaned often enough, no delivery service, not enough stores, insufficient variety of flavors, lack of parking facilities, and store not attractive. In the opinion of the manager, surveys of this nature provide some helpful information. However, the wide divergence of opinions expressed frequently was conflicting and did not permit clear-cut conclusions. W.J.C.

298. Dealer Price Education. V. M. RABUFFO, Ice Cream Trade Jour., *43,* 5: 40. May, 1947.

Suggestions are offered to dealers on how to establish retail prices. The price of fountain items, sodas, sundaes, and milk shakes should be about twice their cost. A satisfactory spread in the cost and selling price of ice cream sold in cones may be maintained by a slight reduction in the amount of ice cream served in cones in lieu of a price increase. A cone sold for double the price of a single cone should contain two scoops of ice cream and represent double value.

Dealers are advised to sell hand-dipped ice cream at 10 cents per pint over the price of a factory-filled package. A gross margin of 30 to 35% is suggested for factory-filled packages.

Retail prices should be set so that they will not retard sales. The price must be acceptable to the consumers, must bear the proper relation to prices of competing items, give the merchant a profitable return on his investment, and produce the greatest possible number of inventory turn-overs in any given period. The dealer should be shown that ice cream is a fast turn-over item in relation to most merchandise carried in stores. W.H.M.

MILK

299. **Grade A Milk.** H. A. RUEHE, Univ. of Illinois, Urbana. Milk Plant Monthly, *36,* 6: 30–32. 1947.

Considerable confusion exists in some localities as to what constitutes Grade A milk. The Milk Ordinance and Code formulated by the Public Health Service sets a standard of 50,000 per ml. for raw milk and 100,000 per ml. for raw cream of this grade. For Grade A pasteurized milk and cream, the bacterial plate count must not exceed 30,000 per ml. for milk and 60,000 per ml. for cream. Certain factors in the production of Grade A milk increase the costs of production so that it may not be economical for the small producer to strive to qualify for Grade A milk production.

G.M.T.

300. **Procurement of Quality Milk.** W. W. THOMPSON, Rochester Dairy Co-operative, Rochester, Minn. Milk Plant Monthly, *36,* 6: 103–104. 1947.

Procurement of quality milk depends in part on the efforts and relationships of a large number of people. Several basic principles are involved in carrying out a quality program. There must be an incentive for the producer to do a good job. The quality of the milk must be determined regularly. The producer must be kept informed of the job he is doing. Field service must be provided. These cannot be minimized. Cash premiums for producing quality milk have secured results. Likewise, the giving away of premiums for certain credits has been effective in producing quality milk.

G.M.T.

301. **The Microscope in Controlling the Quality of Farm Milk.** C. S. BRYAN, Dept. of Surgery and Medicine, Michigan State College, East Lansing. Milk Plant Monthly, *36,* 2: 52, 54, 72. 1947.

Microscopic examination may be used in detecting the source of contamination of poor quality milk. The procedure involves selecting a sample at the weighing vat in a sterile tube; preparing a standard smear; staining by

methylene blue, Mallmann's acid stain, Newmann's reagent, or Broadhurst's reagent; and examination of smears with the microscope, using oil immersion. Details of the various techniques are presented in considerable detail.
G.M.T.

302. **Control Practices Used in Supervision of Vitamin D Milk by City and State Milk Sanitarians.** Report of Committee on Applied Laboratory Methods, K. G. WECKEL, Chairman, Univ. of Wisconsin, Madison. Jour. Milk and Food Technol., *10,* 3: 167–171. May–June, 1947.

Vitamin D fortified milk constitutes from 30 to 60% of the total fluid milk sales. It is important to the public that the desired potency be present in all vitamin D milk produced. Studies indicate that one out of every seven dairies produces milk below the desired potency. One out of every 20 samples collected by city sanitarians for bioassay purposes was found to be below standard.

Fortunately, inadequate fortification does not do the harm that could be done by inadequate pasteurization. Consumers as a rule pay a premium for vitamin D fortified milk and must rely upon assurance for labeled potency. In the light of this survey, many sanitarians should re-assay their procedure of supervision of vitamin D milk produced in their areas. H.H.W.

303. **Developments and Sales Possibilities of Homogenized Milk.** C. A. IVERSON, Dept. of Dairy Industry, Iowa State College, Ames. Milk Plant Monthly, *36,* 5: 64, 66, 68. 1947.

Although many developments have occurred within the last decade in the use and distribution of homogenized milk, probably its greatest development is yet to occur. Advantages claimed for homogenized milk are: (a) butterfat is uniformly distributed, (b) milk is uniform, thus appearing and tasting richer, (c) curd tension is reduced, (d) as a beverage it is a superior product, (e) for infant formulas, boiling, filtering and cooling can be eliminated, (f) certain defects are less pronounced in homogenized milk, (g) in the absence of cream there is no temptation for the operator to cut pasteurization temperature of holding time in order to protect creaming ability, and (h) homogenized milk does have sales appeal. On the other hand, homogenized milk does have its disadvantages, such as (a) no cream layer, (b) certain flavor defects, (c) sediment, (d) disadvantages in cookery, (e) cream line formation if process is improperly done, (f) utilization of returns, (g) increased costs, and (h) the homogenizer may be an additional source of contamination. The author believes that the main advantage which homogenized milk has is that people prefer it as a beverage to non-homogenized milk, and he concludes that homogenization is one of the forward-looking phases of the dairy industry.
G.M.T.

304. What to Check and How to Check on Short-Time Pasteurizers.

A. H. Rishoi, Cherry-Burrell Corp. Milk Plant Monthly, *36*, 5: 34–36, 38–41. 1947.

Attention is called to the many means and devices used to insure compliance with health department regulations and to some of the safeguards which have been built into the design and construction of short-time units. A short-time pasteurizer consists, essentially, of six parts: a float control tank, a regenerator section, a heater section, a holder tube, a flow diversion valve, and a cooler section. The general regulations which should be checked by the sanitarian with respect to milk flow are: (a) the pasteurized milk must be under greater pressure than the raw milk contained in the unit, (b) the milk pump must be sealed so the specified capacity is not exceeded, (c) a restriction is placed in the diversion flow line in order that the back pressure on the milk pump and milk in the holder pump will be the same during diversion, (d) the holding tube should slant upward continually from the point of entrance of the milk to the diversion valve, and (e) drainage to the float tank when the pump stops or when the raw milk line is disconnected from the regenerator outlet must be automatic.

Tests for measuring the time required for milk to pass through the holding tube or providing for injecting a test solution through a stopcock at the entrance and means for drawing liquid through a similar arrangement just ahead of the flow diversion valve have been devised. These test liquids involve a color solution or salt solution. The actual time for the solution to pass through the holding tube may be determined by observation or by electric current. Capacity of the unit is measured by the time required to fill a 10-gallon container. The function of the flow diversion valve is the very heart of the time-temperature control system. Tests on the flow diversion unit involve four steps, which include checking the recording thermometer and adjusting the controller by use of liquids at various temperatures. The paper is unusually informative and helpful to both operators and inspectors of short-time pasteurizers. G.M.T.

305. Experiences Using the Short-Time High-Temperature on Milk, Cream, Buttermilk, Cocoa and Cottage Cheese. M. M. Kloser, Bowman Dairy Co., Chicago, Ill. Milk Plant Monthly, *36*, 3: 38, 40–41, 86. 1947.

The high-temperature short-time pasteurizer has been used successfully in processing milk, cream, buttermilk, cocoa, and milk for cottage cheese. Temperatures employed for different products vary, depending upon the products being processed. Homogenized milk was pasteurized at 170° F. for 16.5 seconds, while the skim milk intended for buttermilk manufacture was heated to 185–190° F. Satisfactory results were obtained on the bases of bacteria, phosphatase, cream volume, and flavor. Greatest difficulty with the use of the high-temperature short-time pasteurizer was with gaskets of synthetic rubber; these had to be replaced more often than the natural rubber gaskets, which had given 16 months of continuous service. G.M.T.

306. Milk Pail. R. J. GOODEN. U. S. Patent 2,422,541, June 17, 1947 (2 claims). U. S. Patent Office Off. Gaz., *599*, 3: 500. 1947.

A milk pail is described with a removable strainer and hinged cover. R.W.

SANITATION AND CLEANSING

307. Sanitary Standards for Dairy Equipment. E. H. PARFITT, Evaporated Milk Assoc., Chicago, Ill. Natl. Butter and Cheese Jour., *38*, 7: 48. July, 1947.

The Subcommittee on Sanitary Standards of the Dairy Industry Committee was appointed to collaborate with the International Association of Milk Sanitarians and the U. S. Public Health Service in developing standards of sanitary performance of processing equipment. Progress has been made, as evidenced by recommendations for fittings, published in the Jour. Milk Technol., Jan.–Feb., 1946, and for storage tanks, *ibid.*, May–June, 1946. Standards for transportation tanks, homogenizers, pumps, storage tanks, and farm milk utensils are being formulated. The success of these undertakings depends on the support of milk sanitarians and regulatory groups and on the education of buyers to specify 3A sanitary standards. W.V.P.

308. Sanitation and Plant Efficiency. C. E. WHITE, Kraft Foods Co., Chicago, Ill. Milk Plant Monthly, *36*, 2: 34–39. 1947.

Sanitation involves the struggle between man and the lower forms of life, such as yeast, bacteria, molds, insects and rodents, which may infest dairy plants. In checking a plant for sanitation, the operator should observe: plant and equipment cleanliness, plant operating methods, orderliness or plant housekeeping, and the condition of the building, premises, and plant equipment. The selection and training of plant personnel, and the proper selection of cleaners, sterilizers, and cleaning aids are of foremost importance. Fundamentally, cleaners serve three purposes—the dissolving of dairy soil, the reduction of interfacial tension between cleaning solution and soil, and the softening of the water through the formation of soluble compounds. Scheduled cleaning, *i.e.*, cleaning the equipment at the proper time, entails four fundamental preventive measures: (a) rinsing equipment between batches, (b) cooling the pasteurizer before and during emptying, (c) elimination of steam leaks, and (d) rinsing equipment immediately after emptying to remove all foam and dairy soil. Insect control is another factor in attaining plant sanitation; this involves eliminating breeding places, insect proofing the building, and the correct use of insecticides. Rodent control also is necessary and includes elimination of breeding places, rodent proofing the building, and trapping. G.M.T.

309. Bottle Washing Problems. D. H. Jacobsen, Cherry-Burrell Corp., Chicago, Ill. Milk Plant Monthly, *36,* 6: 42–44, 46–48. 1947.

The principal function of the bottle washer is to produce clean and sterile bottles. The problems in soaker bottle washing, according to their relative importance, are efficient sterilization, complete cleaning and bright appearance, prevention of etching of glass or color labeling, prevention of scale on the washer, and smooth mechanical operation or lubrication. Certain temperatures and concentration relationships must be maintained in bottle washing. Different states and municipalities have various standards for sterilization of bottles. Until uniform standards are established, milk plant operators should follow local standards on caustic concentration and then choose bottle washing compounds which provide the maximum in bottle cleaning efficiency. G.M.T.

310. Quaternary Ammonium Compounds in the Dairy Industry. M. E. Davis, Winthrop Chemical Co., Weston, Mass. Milk Plant Monthly, *36,* 3: 48, 50–52. 1947.

Quaternary ammonium compounds, of which "Roccal" was the first, have been introduced to the dairy industry with favorable results. The properties claimed for these compounds are: (a) rapid and complete killing action against bacteria even in low concentrations, (b) practically nonpoisonous, (c) non-irritating to the skin, (d) odorless, (e) non-corrosive, (f) great stability, and (g) inhibit bacterial growth over long periods of time. In obtaining maximum efficiency with the use of these compounds, the following suggestions are helpful: clean all equipment; dip all small parts in disinfecting solution before assembly; leave loose all joints, pipe connections, dead-end tees, and valves in order that all surfaces may be contacted by the solution; and pump through the entire system a solution containing 200 p.p.m. Specific directions are given for the use of quaternary compounds in sanitizing milk cans, milking machines, and dairy utensils.

G.M.T.

311. A Uniform System of Milk Inspection. W. D. Tiedeman, Bureau of Milk Sanitation, New York Dept. of Health, Albany, N. Y. Milk Plant Monthly, *36,* 3: 72, 74, 76. 1947.

A complete lack of uniformity exists in the system of milk inspection, not only by the municipalities but among various states. Attempts at securing legislation requiring a uniform inspection system in New York were not successful in 1944 and again in 1945. If a reasonably efficient and uniform sanitary control over country sources of milk supply was maintained, duplication and much ill-feeling caused by stringent inspection in some areas and laxity of inspection in others would be eliminated. Indications are that in the future agreements between departments of health, agriculture, markets, and the dairy industry will be effected. G.M.T.

312. Essentials of an Effective Farm Dairy Sanitation Program. P. W. SCHERSCHEL, Laboratory and Field Service, Pure Milk Assoc., Chicago, Ill. Milk Plant Monthly, *36,* 6: 105–106. 1947.

An effective farm dairy sanitation program involves good cooperation between the receiving room, trucker, fieldman, and the producer. The fieldman has certain responsibilities in suggesting to producers ways and means of improving the quality of their milk. Producers as a rule want to market a good quality product, keep healthy cows, be clean, follow correct milking procedures, and control temperatures. Detailed directions for cleaning utensils, milking, and the care of the milk house and surroundings are given. G.M.T.

313. Duties of a Fieldman. W. J. BRAKEL, Dept. of Dairy Husb., Ohio State Univ., Columbus. Milk Plant Monthly, *36,* 4: 48, 50. 1947.

The duties of a fieldman are to satisfy his employer, to represent the firm by which he is employed, and to render service to the dairy producer. He often is the only representative of the organization purchasing the milk and as such should bring to the producer a favorable opinion concerning the plant. Retaining the goodwill of producers and increasing production are not difficult if the fieldman has proper attitudes and approaches a patron in a tactful, businesslike manner. Appealing to pride is an effective means of stimulating producers to milk quality; as a last resort, the producer may be advised that the market requires that certain changes be made in order to help in producing a higher quality product. The fieldman especially must be in close contact with receiving room operations, such as weighing, Babcock testing, and quality tests. Cans returned to the producers must be sanitary. The fieldman should spend sufficient time at the plant conducting quality tests and keeping records to enable him to have complete confidence in their accuracy. He should function as a service man, creating interest and enthusiasm for efficient production of high quality milk. He must be interested in his work, in its importance, and in keeping up-to-date with improved methods. G.M.T.

314. The Use of DDT and Other Methods of Insect Control in Dairy Plants. L. S. HENDERSON, Bureau of Entomology and Plant Quarantine, Dept. of Agr., Washington, D. C. Milk Plant Monthly, *36,* 3: 54–56, 60. 1947.

Preventive measures in controlling insects around food establishments may be even more important than the application of insecticides. Sanitation and rigid and thorough house-cleaning or plant-keeping are highly important. Sanitation should not end inside the plant but should include the premises as well. DDT is an extremely useful insecticide; however, there has been a great deal of hesitancy in recommending its use for fly control in rooms where food is processed or handled because the insects may, in the process of being destroyed, get into the equipment or food. DDT likewise is effective in roach control. A heavy roach population may appear a few days after its application because of the stimulation effected by DDT in bringing them out of their hiding places. If roaches still are present after 2 weeks, incomplete coverage usually is indicated. In using insecticides the importance of sanitation must not be minimized. G.M.T.

315. Insect and Rodent Control in Dairy Plants. F. A. KORFF, Bureau of Food Control, Baltimore City Health Dept. Milk Plant Monthly, *36,* 4: 98, 100. 1947.

The dairy industry has been fairly successful in the fight against microbial contamination of their products. Attention today is being given to the elimination of infestations from insects and rodents. Like bacteria, insects and rodents need food, water, and warm environment. By eliminating breeding places through sanitation and plant housekeeping, a great deal can be accomplished in keeping pests at a minimum. Recently emphasis has been placed on the importance of plant housekeeping, rather than on trapping and killing. The technical method of control consists of sealing up all openings where insects and rodents may enter and eliminating harboring places throughout the building. The biological method involves the reduction and elimination of all hiding and breeding areas. Effective results can be obtained by proper training of plant personnel and by the relocation of equipment so as to aid in cleaning and inspection operations. Sanitation is of utmost importance in the control of all dairy plant pests. G.M.T.

316. Insect and Rodent Control in the Plant. E. M. SEARLS, Sealtest, Inc., N. Y., N. Y. Milk Indus. Found. Assoc. Bul., *39,* 12: 350–354. March, 1947.

Insect and rodent control involves not only destruction of the pests but primarily preventing their entrance into food-handling establishments. The statement is made that about 80% of all convictions by the Federal Security Agency for adulteration of product published in the July, 1946, list were based on presence of insects or rodents or on that "might have been" clause.

At the present time there seems nothing more available or desirable than the insecticide DDT, which permits virtual proofing of a plant against insect occupation for long periods of time. Nothing approaching DDT for insects is available for rodents. It is questionable if poisoning rodents is advisable. Exclusion by rodent proofing is the first line of defense, with the old clap-trap style rat and mouse trap for those that get in. E.F.G.

317. **New Chemicals for Insect Control on the Farm.** E. M. SEARLS, Sealtest, Inc., N. Y., N. Y. Milk Indus. Found. Assoc. Bul., *39,* 9: 240–242. Feb., 1947.

Sanitation comes first in insect control. Have clean barns, clean premises, and clean, well-tended animals; then the application of insecticides will finish the job of insect control. Probably the most useful insecticide is wettable DDT applied with a knapsack-type sprayer according to the instructions of the manufacturer of the insecticide. Neither DDT nor any of the rest of these new insecticides can take the place of sanitation. E.F.G.

318. **Ten Common Causes of Excessive Sediment. Part 1. Importance of Keeping Cows Clean.** C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36,* 2: 64, 66. 1947.

Thousands of service calls on sediment trouble indicate that the ten most common causes of poor sediment tests at the receiving platform are: (a) cows, (b) milk cans, (c) improper use of filter disc and strainer, (d) failure to rinse strainer when filter disc is changed, (e) carelessness in storage of milk can lids, (f) milking machine suction cups allowed to get close to floor or bedding when being attached to the cow, (g) improper technique in pouring milk into strainer, (h) sediment in rinse water, (i) jarring lock strainer, and (j) single face discs placed in strainer with the gauze side up; the gauze should be down with the cotton side up. G.M.T.

319. **Ten Common Causes of Excessive Sediment in Milk. Part 3. Proper Care of Milk Can Lids.** C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36,* 4: 86, 88. 1947.

Careless handling and storing of milk can lids has been found to be one source of excessive sediment in milk. Fine dark specks found in milk were traced to pieces of bark falling from a tree into unprotected can lids which later were used without cleansing. G.M.T.

320. **Ten Common Causes of Excessive Sediment in Milk. Part 4. Filter Disk Improperly Placed in Strainer.** C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36,* 5: 82–83. 1947.

Fifty per cent of 2,700 milk producers who had milk returned because of undesirable sediment tests were not placing the filter disc into the strainer properly; thus some of the milk by-passed the filter and was not strained. Every plant manager, fieldman, and milk hauler should learn how to place the disc into a strainer correctly and pass this information on to each of the milk producers. Both hands should be used in placing the disc. Unless the edges fit properly, the baffle plate or dome crumples the sediment disc, preventing a perfect seal. Dents in the bottom of strainer will cause similar by-passes. G.M.T.

321. Ten Common Causes of Excessive Sediment in Milk. Part 5. Failure to Rinse Strainer when Changing Disk. C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36,* 6: 98–99. 1947.

Rinsing the strainer when changing the disc has been found to be a means of eliminating some of the sediment from milk. Unless this is done, some of the sediment taken out of the milk may lodge on the strainer under the new disc and be washed into the milk. G.M.T.

MISCELLANEOUS

322. Dairy Waste Saving and Disposal. W. A. DEAN, JR., Bowman Dairy Co., Chicago, Ill. Milk Plant Monthly, *36,* 5: 42–48, 50. 1947.

Dairy waste disposal is an old problem in which increasing interest is shown today. Reduction of waste in a plant to the minimum is financially beneficial, as well as a means of avoiding possible lawsuits. Plants have been sued for devaluation of property, injury of livestock, discomfort, loss of health and well-being, destruction of fish and wild life, destruction of plant and plankton life, and general nuisance. Dairy wastes may be classified as follows: (a) waste due to inefficient processing equipment, methods, or operation; (b) waste of whey, buttermilk, skim milk or other by-products; (c) waste of spoiled products; and (d) wastes due to rinsings and wash water. Methods of keeping waste to a minimum and the various systems involved in treatment of necessary wastes are described fully. Dairy waste sampling and testing followed by determination of the causes of excessive losses aid in reducing losses. G.M.T.

323. Effect of Stabilizers on Frozen Cream. R. W. BILL, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Dept. of Agr. Jour. Milk and Food Technol., *10,* 3: 149–157. May–June, 1947.

The use of a stabilizer which would make it possible to obtain thawed cream resembling bottled whipping cream was investigated. The stabilizers used were emargol and fruit pectin. These compounds, when added to raw cream, appeared to help conserve the physical stability of the pasteurized cream during low temperature storage. Emargol tended to decrease the tendency of thawed creams to oil off, without changing the viscosity. Pectin improved the consistency and increased the viscosity of the thawed cream without the tendency to form a cream plug. The differences between the control samples and those which contained a stabilizer were more pronounced when the freezing and storage temperature was – 15° C. than when it was – 29° C.

Emargol and pectin were valuable in stabilizing the cream. However, they were not so important as the size of the fat globules, avoidance of clumping, rate of freezing, and storage temperature. H.H.W.

324. Butterfat and Solids-not-fat. Their Relation to Plant Control and Their Implication in Plant Operations. L. C. THOMSEN, Univ. of Wisconsin, Madison. Milk Indus. Found. Assoc. Bul., *39*, 10: 257–266. March, 1947.

The author suggests that losses be "departmentalized" and not taken merely as over-all plant losses. This is to fix responsibility. If two methods of expressing losses are used, such as per cent of the fat lost and per cent of product lost, then one method may be used as a check against the other and may assist in identifying the point of loss.

In calculating fat losses it is important that the sample in the receiving room be accurate and that the analysis of the original milk samples, as well as the fat content of the various products, yield as near true fat contents as possible. For some products the usual Babcock method is not satisfactory, and a modification must be used. In that part of the milk skimmed, the usual fat loss in the skim milk from 5.0% milk will amount to 1.3% of the fat, while from 3.0% milk it will amount to 2.15%. In working out the losses on the basis of the weight of product, accurate weighing or measuring devices must be provided, containers must have the correct volume, and suitable conversion factors must be used.

The total "paper" losses in ideally operated plants selling only fluid milk and cream will amount to approximately 1.65%, of which slightly less than three-fourths can be assigned to the fluid milk part of the operation and slightly over one-fourth can be charged to the fluid cream operation. The percentage of loss may vary widely from the above if corresponding testing procedures are not used. E.F.G.

325. Plant and Accounting, Their Relation and Contribution to Efficient Management. H. LAGONI, Borden's Dairy Delivery, San Francisco, Calif. Milk Indus. Found. Assoc. Bul., *39*, 10: 281–287. March, 1947.

It is not difficult to determine when costs are too high but it is important that the cause of the high cost be found and the extent to which it is high actually determined. The various items of cost—such as plant, labor, equipment, materials, supplies, power, steam, refrigeration, waste, and container loss—should be determined on the basis of appropriate units and these costs compared with previously established reasonable standards. This fact-finding operation will furnish the accounting data necessary to a better management job. E.F.G.

326. The Relation of Plant Operations and Accounting to Efficient Management. R. J. Ramsey, Ramsey Laboratories, Cleveland, Ohio. Milk Indus. Found. Assoc. Bul., *39,* 10: 267–280. March, 1947.

Periodical examination of the records being kept will show some which can be dispensed with and others which are needed. Butterfat accounting is considered to be of first importance, and it should not be difficult to keep these losses below 1% of the fat purchased. Among the procedures recommended for fat checking are the use of a drip sampler for total fat, the use of an automatic sewage sampler, accurate fat standardization of all products, correct weight of product in all containers, and collecting drip, rinsings, and foam for later separation. The per cent of fat in skim milk should be figured at 0.1% as shown with the Mojonnier test.

Bottle trippage is checked by weighing broken glass at critical points and keeping the bottle deposit on wholesale bottles as high as possible. Unless the market is almost ready for paper, it is suggested that substantial savings may be effected with lightweight 48 mm. bottles. Keeping cottage cheese yields up to 16–18 lb. per 100 lb. of skim milk, careful checking of ice box inventory and steam costs, larger use of latent heat equipment, and higher back pressure in the refrigeration plant are important methods of increasing efficiency.

A point to be kept in mind is that it costs approximately five times as much to cool milk one degree as it does to heat it one degree. With S.T.H.T. pasteurization, the use of regeneration may decrease the use of steam to the point that the batch method would require 365% of the S.T.H.T. requirement and in refrigeration the batch method would require 180%, with the S.T.H.T. requirement considered 100%. Industrial water treatment is a source of very substantial savings. E.F.G.

327. How a Safety Program Can Save You Money. E. G. Quesnel. The Borden Co., N. Y., N. Y. Milk Indus. Found. Assoc. Bul., *39,* 12: 355–360. March, 1947.

The total cost of an accident is on an average four times the direct cost. The all-industry accident rate compiled for 1945 by the National Safety Council shows accidents per million hours worked to be 13.53; for the food industry it was 22.80, while for the dairy products industry it was 26.02.

Accidents in the dairy industry apparently occur almost twice as often as in all industry. The Milk Industry Foundation started a vehicle accident contest Sept. 1, 1939. Of 166 fleets enrolled for the year, only 4 reported no accidents and 36 had less than 5 accidents. The contest was continued each year and the contest ending Sept. 1, 1946, showed 209 fleets enrolled, 19 having no reportable accident and 63 having less than 5 accidents.

A contest for plant workers started in 1941, with 121 companies enrolled, showed 12 with no accident the first year and 52 with less than 5 accidents. In the contest ending Sept. 1, 1946, 21 companies experienced no reportable accident and 90 companies had less than 5 accidents. The contests call attention to safe operating rules and regulations, supply instructions for setting up a safety department in each company, and in many ways draw attention to the importance of safe practice and procedure. E.F.G.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

328. Places Where Practice in Making Laboratory Examinations of Dairy Products Has Not Caught Up with Research Findings. R. S. BREED, New York State Expt. Sta. (Cornell University), Geneva. Milk Indus. Found. Assoc. Bul., *39,* 15: 431–447. May, 1947.

The various conditions under which milk is produced, processed, contaminated, held in storage, and modified by addition of such materials as chocolate, vitamin D, etc., make for wide variation in the types of bacteria which may be present. A laboratory using only one temperature of incubation many times will be in error unless additional plates at other temperatures are used or if agar plates are not checked with microscopic preparations. Curves are presented showing in general a higher colony plate count on plates incubated at 48 hr. at 32° C. than for the same length of time at 35° C. or 37° C. A temperature of 32° C. is recommended.

The problem of coliform standards is suggested as one for individual enforcement officers, since reasonable standards will vary from one milk area to another. The Montreal milk supply is reported to meet standards of less than 1 coliform organism per ml. of milk, while the upstate New York inspectors have not found it advisable to enforce a standard more severe than 10 coliforms per ml. Long storage periods to which pasteurized milk frequently is subjected cause bacteriological conditions about which more information is needed and upon which more research should be done.

E.F.G.

329. L'analyse bacteriologique des laits concentrés sucrés. Presence de staphylocoque pathogènes dans certains d'entre eux. (Bacteriological Analysis of Sweetened Condensed Milks. Presence of Staphylococcic Pathogens in Certain of Them.) R. BUTTIAUX AND L. LESNE, Pasteur Institute, Lille, France. Le Lait, *27,* 263–264: 121–131. March–April, 1947.

In 1945, samples of sweetened condensed milk of foreign origin were found to produce serious illnesses among infants. Details are given of the bacteriological methods used in studying samples of this product and the results secured. Cans were examined for swelling, for total and *B. coli* count, and for the presence of putrid organisms, anaerobes, yeasts, ropy organisms, and salmonella.

In isolating and identifying the staphylococci, Gram stains were made of cultures obtained in lactose broth. These were cultured on nutrient agar containing tryptone and glucose. After 24 hr. at 37° C., typical golden and white colonies were picked for further study. Pathogenic strains were distinguished by color of colony, coagulation of blood serum, haemolysis, fermentation of mannitol, and production of violet color on lactose gentian-violet agar.

Of 66 different samples examined, 20 showed the presence of staphylococci, and 16 of these were pathogenic. Numbers varied from 100 to 100,000 per l., but types rather than numbers appeared more important in determining the severity of the sickness which occurred. Contamination of these products probably took place during processing rather than from organisms present in the raw milk surviving heat treatment. O.R.I.

BUTTER

330. **Milk Fat Concentrate Process.** H. C. Horneman, R. V. Hussong, S. N. Quam, and B. W. Hammer. (Assigned to Sugar Creek Creamery and Cherry Burrell Corp.) U. S. Patent 2,423,834, July 15, 1947 (6 claims). U. S. Patent Office Off. Gaz., *600,* 3: 382. 1947.

Cream under reduced pressure is subjected to the action of steam, then centrifuged to yield milk fat, mixed with water and salt, and finally suitably cooled and stabilized to produce a product like butter. R.W.

CHEESE

331. **Flavor Development in Cheddar Cheese.** A. C. Dahlberg and F. V. Kosikowsky, Cornell University, Ithaca, N. Y. Natl. Butter and Cheese Jour., *38,* 8: 44. Aug., 1947.

The formation of the flavor compounds in Cheddar cheese has never been fully explained by scientific knowledge. It is known that during ripening carbon dioxide is formed, lactose disappears, pH decreases and then increases, protein decomposes into simpler nitrogenous compounds and amino acids, and volatile fatty acids are formed. These changes are caused by the combined actions of bacteria and of enzymes added with rennet extract. Science has not determined any exact relationship of protein decomposition and development of volatile fatty acids to intensity of flavor production. It is known, however, that high temperatures of curing tend to stimulate these changes that are observed as flavor is produced in cheese made from pasteurized milk and from raw milk of high quality. New methods must be used to study these problems. W.V.P.

332. Étude comparée des fromages de Livarot et de Pont-L'Évêque. (Study Comparing Livarot and Pont-L'Eveque Cheeses.) C. Hurel and G. Mocquot. Le Lait, *27*, 263–264: 131–141. March–April, 1947.

The characteristics of, and to a limited extent, the methods used in manufacturing these two varieties of cheese are described. Both types are products of the district of d'Auge in Normandy.

Livarot, a small cylindrical cheese about 12 cm. in diameter and 5 cm. tall, requires much care during the 3 months of ripening. It is made from partially skimmed milk, the finished cheese containing 30 to 40% fat in the dry matter. Acidity develops rapidly during manufacture, although the development of proper flavor and texture is dependent upon the growth of a surface coat containing yeasts, molds, and proteolytic bacteria. Pont-L'Évêque, a square cheese with dimensions of 11 cm. a side, is made from whole, fresh milk with rennet and is ready for consumption in 4 to 6 weeks. An orange-tinted slime develops on the surface of the cheese during ripening.

Graphs are presented showing comparative rates of lactic acid development, reduction in weight and moisture content during ripening, variations in pH during ripening, and rate of salt penetration. O.R.I.

CHEMISTRY

333. Calcium Content of Fluid Milk. C. H. Robinson, J. Hlynka, F. A. Herman, and J. W. McArthur, Dept. of Agr., Ottawa, Canada. Canad. Public Health Jour., *38*, 5: 236–239. May, 1947.

The calcium content of fluid milk was determined at weekly intervals for 1 year (Oct., 1944–Oct., 1945) from representative dairies in Ottawa, Ont., Kentville, N. S., and Summerland, B. C., and continuous line charts were plotted from the results. There is a suggestion of a midwinter and midsummer fall in the curves. Mean values for the three localities were 117, 112, and 139 mg. Ca per 100 ml. milk, respectively, indicating a highly significant difference. Milks from individual dairies in Ottawa yielded nearly identical mean values, but there was a difference between dairies in the other two areas.

Other areas might yield calcium values lower than those found in the Ottawa data. If an error of ± 20% is too great for purposes of dietary survey, the mean calcium content of the fluid milk for the area under survey should be determined. O.R.I.

334. Milk Studies. I. Some Vitamin and Trace Elements Found in the Colostrum of the Dairy Cow, Beef Cow, and Swine. R. W. Luecke, C. W. Duncan, R. E. Ely, E. Jones, A. J. Greene, and

C. Tull, Michigan State College, East Lansing. Arch. Biochem., *13,* 2: 277. May, 1947.

Essentially the same concentrations of iron, copper, and cobalt were found in the colostrum milk of the beef cow and of the Jersey and Holstein dairy cow. The iron and copper contents of colostrum from both dairy and beef cows were found to be higher than values reported for milk. Carotene and vitamin A contents of colostrum milk from the Jersey cow averaged 0.347 and 0.401 mg./100 ml., respectively, as compared with 0.100 and 0.052 mg./100 ml. in Holstein colostrum, and 0.129 and 0.145 mg./100 ml. in colostrum of the beef cow. H.J.P.

335. Recherches sur les variations du taux du lactose dans le lait de vache pendant le travail. (Studies on the Variations in the Lactose Content of Milk of Cattle during Work.) A. Pino. Le Lait, *27,* 263–264: 113–121. March–April, 1947.

Physiological evidence indicates that muscle activity and the production of lactose in milk are competitive in their demands upon stored sources of energy in the animal organism. The possibility that cows which were used as work animals would yield milk containing smaller percentages of lactose was investigated. Lactose analyses were made on milk samples from 50 cows at rest, after 1, 2, 3, and 4 hr. work, and after 1, 2, 3, 4, and in some cases 5, 6, and 7 hr. rest. Only ten of the cows were accustomed to working.

The lactose content of the milk of both groups declined during work and was restored by rest, the diminution being more marked in the group not accustomed to working. However, the lactose content of the "working" cows was low at all times, in some cases being less than 2%. O.R.I.

336. Composition of Buffalo Milk Fat. C. P. Anantakrishnan, V. R. Bhalerao, and T. M. Paul, Indian Dairy Research Inst., Bangalore, India. Arch. Biochem., *13,* 3: 389. June, 1947.

The influence of feeding oils and fats on the composition of buffalo milk fat was determined. When a basal ration was supplemented by coconut oil and its hydrogenated product, a significant increase in the lauro-myristic group and a marked fall in palmitic acid occurred. Cottonseed oil supplementation reduced the amount of the lower saturated acids up to palmitic acid and increased the content of oleic and stearic acids. Ingestion of groundnut and sesame oils led to an increase in the oleoglycerides of the resulting milk fat with a corresponding decrease in palmitoglycerides. The selective adsorption of lauro-myristic glycerides by the mammary gland indicates that the mechanism of secretion of fat by buffaloes is almost similar to that of cows. H.J.P.

337. Direct Estimation of Leucine (Application to Milk and Certain Other Foodstuffs). A. Z. HODSON AND G. M. KRUEGER, Pet Milk Co., Greenville, Ill. Arch. Biochem., *12*, 3: 435. March, 1947.

The leucine content of dry skim milk and dry whole milk was determined by two microbiological methods. Direct assays of unhydrolyzed samples of skim milk conducted with a "leucineless" mutant of *Neurospora crassa* averaged 4.01% leucine; dry whole milk averaged 3.16% leucine. Acid hydrolyzed samples of skim milk assayed with *Streptococcus faecalis* averaged 4.08% leucine, and dry whole milk averaged 3.09% leucine.

H.J.P.

338. Preparation and Properties of Serum and Plasma Proteins. XIII. Crystallization of Serum Albumins from Ethanol-Water Mixtures. E. J. COHN, W. L. HUGHES, JR., AND J. H. WEARE, Harvard Medical School, Boston, Mass. Amer. Chem. Soc. Jour., *69*, 7: 1753. July, 1947.

Long chain aliphatic alcohols of 5 or more carbon atoms permitted the reproducible crystallization of serum albumins from ethanol-water mixtures. Bovine serum albumins crystallized readily over the following range of solvent composition: ethanol volume per cent at 25°, 33 to 40; mole fraction, 0.12 to 0.163; ionic strength, 0.32 to 0.65; pH, 5.3 to 5.7; temperature, + 5 to − 10° C.; and 10 to 20% protein concentration. Constants defining the properties of purified serum albumins are tabulated. H.J.P.

339. Three Sources of Amino Acids for Niacin Assay. A. R. KEMMERER AND F. SHAPIRO, Univ. of Arizona, Tucson. Indus. and Engin. Chem., Analyt. Ed., *19*, 5: 358–359. May, 1947.

In the Snell-Wright microbiological assay for niacin, acid-hydrolyzed casein has been used as a source of amino acids for the assay organism. This comparison of trypsin-hydrolyzed casein and charcoal-treated peptone with acid-hydrolyzed casein indicates that any of these sources of amino acids will give reliable results. Some characteristics of each amino acid preparation are discussed. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

340. Flavor and Color of Sweetened Condensed Milk. G. C. NORTH, Beatrice Foods Co., Chicago, Ill. Milk Plant Monthly, *36*, 7: 38. 1947.

Sweetened condensed milk will possess a fine fresh milk flavor over an extended period if the product is made from high quality milk in properly sanitized equipment, free of copper contamination, and heated sufficiently to inactivate the enzyme lipase. Rancidity and tallowiness are two possible flavors of sweetened condensed milk. Progressive darkening is quite noticeable when dextrose or corn sirup is used as part of the sweetening agent and the product is held at a high temperature. Cool storage is essential in order to retard the development of a dark color. G.M.T.

341. Sources of Sediment in Bulk Sweetened Condensed Milk. C. A. DAY, The Bennett Creamery Co., Ottawa, Kans. Milk Plant Monthly, *36,* 8: 66, 68, 70. 1947.

Sediment or extraneous matter may get into condensed milk from any of the following sources: (a) poorly cleaned equipment, (b) rust and sediment from water and steam pipes, (c) washing powders, (d) scouring pads, steel wool, metal sponges, brushes, and abrasives, (e) empty bungless barrels, (f) insects and rodents, (g) sugar sacks, and (h) sugar. G.M.T.

342. Observations on the Viscosity of Sweetened Condensed Milk. B. H. WEBB, Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Dept. of Agr. Milk Plant Monthly, *36,* 7: 48, 50, 52. 1947.

Viscosity is one of the important indications of quality in sweetened condensed milk. The more heat to which sweetened condensed milk is subjected after condensing, the more viscous will the milk become. The viscosity increases with temperature and time of holding. Lower forewarming temperature (160° F.), adding cane or beet sugar at the close of the concentration process, avoidance of overheating toward the end of the process, avoidance of super-heating, cooling properly after condensing, and storing at a low temperature (60° F. or under) tend to keep the viscosity of the final product at a minimum. G.M.T.

343. Changes in the Essential Amino Acid Content of the Proteins of Dry Skim Milk on Prolonged Storage. A. Z. HODSON AND G. M. KRUEGER, Pet Milk Co., Greenville, Ill. Arch. Biochem., *12,* 1: 51. Jan., 1947.

The essential amino acid content of freshly processed spray-dried skim milk was compared with that of dry skim milk stored at room temperature for 51 months in loosely covered containers. Microbiological assay showed that only those samples which had become dark and unpalatable had marked losses of arginine, histidine, lysine, and methionine. Insignificant differerences in amino acid composition were noted when old samples retained their white color, fresh taste, and appearance. H.J.P.

344. **The Manufacture of Cultured Buttermilk.** W. I. TRETSVEN, Dairy Advisory Service, Chicago, Ill. Milk Plant Monthly, *36,* 7: 24–26, 28. 1947.

The methods of making cultured buttermilk on a large scale are discussed. This body, whey separation, and presence of gas can be overcome by proper processing and handling. Stabilizers are suggested for increasing viscosity and preventing whey separation. Flavor may be enhanced by addition of 0.2% sodium citrate to the skim milk before pasteurization, and by addition of cream and a small amount of salt. G.M.T.

345. **Manufacturing Cultured Sour Creams, Including a Nonperishable Cultured Sour Cream.** W. J. TRETSVEN, Dairy Advisory Service, Chicago, Ill. Milk Plant Monthly, *36,* 8: 24–26, 40. 1947.

Producing a good cultured sour cream consistently day after day is a problem. The manufacturing procedure involves using a cream containing 15–25% fat, pasteurizing at 180° F. for 30 min., homogenizing at 3,000 lb. pressure at the pasteurization temperature, cooling to 70° F., inoculating with 0.5% culture, ripening from 10–18 hr., and then packaging. Viscosity sometimes is increased by the addition of spray-dried nonfat dry milk or concentrated skim milk. Rennet extract sometimes is used where homogenizing is not practiced. Gelatin (0.3%) may be used to increase viscosity, prevent whey separation, and impart other desirable qualities to the cultured cream. Other stabilizers may be used to prevent whey separation. G.M.T.

346. **The Utilization of Whey.** B. H. WEBB AND E. O. WHITTIER, Division of Dairy Research Laboratories, U. S. Dept. Agr. Milk Plant Monthly, *36,* 8: 88, 90, 92. 1947.

The authors have surveyed the field and present graphically, giving brief description and citations, 25 different ways in which whey may be utilized. G.M.T.

347. **What Can Be Done With Whey?** M. D. SANDERS, Swift & Co., Chicago, Ill. Milk Plant Monthly, *36,* 7: 44, 46. 1947.

Tremendous quantities of whey go to waste each year because of lack of means of economical utilization. The solution to the whey problem would be a large commercial outlet for the whey solids and a simple and economical means of processing the whey suitable for small cheese plants handling 10 to 20 thousand pounds of milk per day. As yet no outlet has been developed which will absorb the cost of transportation of raw whey or processing at the source. Numerous methods of utilizing whey in various by-products are given, but as yet these dispose of only a small percentage of the whey available. G.M.T.

DISEASES

348. The Resistance of Chronic Staphylococci Bovine Mastitis to Massive Penicillin Therapy. E. C. McCulloch. Amer. Jour. Vet. Res., *8*, 27: 173–177. April, 1947.

Treatment of 10 quarters of 7 cows chronically infected with *Staphylococcus aureus* served to test the efficacy of massive, repeated infusions of penicillin. Intramammary infusion of a total of 400,000 units per quarter, given in 4 infusions of 100,000 units at approximately 12-hr. intervals after each of 4 successive milkings, was followed by only a temporary reduction in number of staphylococci shed in the milk of treated quarters. There was no evidence of irritation due to the large amounts of the drug used, and milk yields were not significantly reduced following series of infusions. T.M.L.

349. Milk Fever (Parturient Paresis) as a Manifestation of Alkalosis. A. H. Craige, Jr., and I. V. Stoll. Amer. Jour. Vet. Res., *8*, 27: 168–172. April, 1947.

In a study of 12 cases of milk fever, evidence shows that treatment by calcium gluconate relieves acute symptoms rather rapidly and effectively, but the characteristic hypocalcemic condition often persists for some time. In an effort to supplement the present calcium gluconate treatment and to raise the blood calcium levels more rapidly, Chlor-Ethamine was given orally after the animals had regained locomotion. There is some indication that this treatment may be very useful, especially on animals that are subject to frequent relapse after an apparent cure.

Although hypocalcemia is a characteristic symptom of milk fever, it probably is not the fundamental physiologic defect; however, it appears to be secondary to a parturient alkalosis. T.M.L.

350. Vibronic Abortion in Cattle. W. N. Plastridge, L. F. Williams, and Dorothy Petrie. Amer. Jour. Vet. Res., *8*, 27: 178–183. April, 1947.

The examination of 72 fetuses from slaughtered cows of herds suspected of having vibrionic abortion revealed that 20 animals were positive for the infection. The annual abortion rate in 10 herds infected with "vibrio factus" averaged about 12%. Some evidence indicates that infection with "vibrio factus" also influences conception. Bulls may contract the disease. At the present time the method of detecting the infection by the agglutination test is not so accurate as that for Bangs' disease. T.M.L.

ICE CREAM

351. New Sanitary Practices and Controls. M. G. PEDERSON, El Paso, Tex. Internatl. Assoc. Ice Cream Mfrs., Prod. and Lab. Council Proc., *2*: 7–14. 1946.

Water may become a source of a bad sediment pad in ice cream. Air may be important, especially if the plant is located along an alley, if coal is handled in the plant, or if dusty streets are located near the plant. Air may be checked by filtering plant air through a filter cloth attached to a vacuum cleaner. Sediment may come from any ingredient of the mix, cleaning compounds, equipment, or packages.

The sanitarian looks closely at the hands and clothes of the employee and looks almost as closely at the clean-up job done at the end of the day's work. Flies may be controlled by the use of DDT; a 5% solution in oil or water emulsion will last for 9 days to 6 weeks. For other insects such as roaches and ants, rotenone and fluoride are recommended since DDT is not a cure-all for roaches. Proper plant construction may eliminate many of these pests. The several chemicals used by the Army during the war for control of rats are not effective against mice; nor will the construction of the building keep all of these out. P.S.L.

352. The Future of Concentrated and Dry Ice Cream Mixes. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. Ice Cream Trade Jour., *43*, 6: 68. June, 1947.

Concentrated and dry ice cream mixes were made in large quantities during the last years of World War II. These products would keep for about 4 months at 100° F., but the life of the product could be increased by storing at a lower temperature. Since the war sales to the housewife and to the small ice cream manufacturer are increasing. When sugar and milk solids become more available, the small manufacturer may tend to discontinue the use of dry ice cream mix. In some places city ordinances requiring pasteurization of the reconstituted products before freezing have interfered with sales. Central and South American countries may provide enormous markets for these products.

The future for dry ice cream mix appears bright provided a good quality product is maintained and sales are regulated to insure freshness of the product. Workable regulations and a realistic attitude toward the economy of exportation will help promote the sale of concentrated and dry ice cream mix. W.H.M.

353. Corn Sweeteners, Their Place as an Ingredient in Our Post War Ice Cream. ANONYMOUS. Ice Cream Rev., *30*, 12: 45. July, 1947.

Scientists from many of the leading dairy schools, with a few important exceptions, believe that corn sweeteners have won for themselves a permanent place in our postwar ice cream. Some of the reasons advanced are: (a) Corn sugar and corn sirups in particular definitely improve the body texture of ice cream. (b) They make it possible to build up the total solids of the mix at a reasonable cost with less danger of sandiness. (c) They facilitate the production of the heavier bodied, smooth-textured ice cream so much in demand at the present time. (d) The use of suitable combinations of sweetening agents makes it possible for the ice cream manufacturer to control the firmness and relative sweetness of ice cream. (e) Corn sweeteners normally are a relatively cheap source of solids.

Possible drawbacks to the continued use of corn sweeteners are: (a) Their use complicates the mix-making procedure by introducing one or more additional ingredients. (b) It is inconvenient to handle corn sirups in small plants. (c) Some types of dried corn sirups are difficult to handle because of their tendency to lump and cake during storage. (d) Some may impart an objectionable color or flavor to ice cream. Despite some possible drawbacks to the use of corn sweeteners in ice cream, it generally is agreed that these products permanently will supply a part of the sugar solids in the postwar ice cream. W.J.C.

354. Method of Manufacturing Ice Cream and Product Thereof. B. NELSON. (Assigned to Lanco Products Corp.) U. S. Patent 2,423,613, July 8, 1947 (17 claims). U. S. Patent Office Off. Gaz., *600,* 2: 256. 1947.

An ice cream ingredient consisting of a mixed glycerol diester of a higher fatty acid and an aromatic carboxylic acid is described. R.W.

355. Latest Developments in Hardening Ice Cream. R. H. THOKE, Chicago, Ill. Internatl. Assoc. Ice Cream Mfrs., Prod. and Lab. Council Proc., *2:* 15–18. 1946.

The older methods of hardening ice cream by means of ceiling, wall, and shelf ammonia pipes, with periodic defrosting, are messy and damaging to the building construction and insulation. The blast system of air circulation is objectionable to cooler labor. The unit cooler or blower system is sure to replace the older method because it is efficient, provides quick hardening, and is space saving. Defrosting of this newer system may be accomplished by: (a) intermittent water spray over the coil bank, (b) intermittent recirculation of warm air over the coil bank, and (c) continuous defrosting by recirculation of calcium brine over the coil bank. Cold air direction is well guided by using air ducts or, even better, by using draftless outlets such as anemostats. The use of tunnels at –40 to –50° F. with high air velocity for quick hardening of packaged goods may have serious economic disadvantages. Texture of ice cream is not improved by such treatment. P.S.L.

356. Latest Developments in Hardening Ice Cream. J. B. ORRELL, Philadelphia, Pa. Internatl. Assoc. Ice Cream Mfrs., Prod. and Lab. Council Proc., *2:* 18–23. 1946.

At one time the plant described used for hardening packaged ice cream a conveyor belt 6 ft. wide and 40 ft. long. This was discarded to save space. Skids now are used for carrying the ice cream and it is hardened at – 40° F. In the hardening room, vertical bins are used which permit separation of flavors but which require a 14-ft. ceiling. By this means a room may be cooled by using 26,000 ft. of 1.25-in. coils, where in the past 45,000 ft. of 2-in. coils were used in the same space. A suction header for these coils is placed near the ceiling. This arrangement permitted an increase from 12,145, 2.5-gallon can capacity to 23,000, and from 1,292 to 2,920 sq. ft. of floor space. Much more space may be occupied by ice cream by using the vertical coil. The actual gain was 3.7 quarts per cu. ft. over the old system, or approximately 50%. Snow collecting on the coils easily may be scraped off or even swept off. To install this system, considerable height in the room is required. Rooms are maintained at – 20° F. For hardening brick molds, the molds are cut at – 15° F. Bulk ice cream can be hardened in 8 to 10 hr., although to accomplish this, temporary blowers are placed in the aisles of the hardening room each night. P.S.L.

357. Latest Developments in Hardening Ice Cream. H. BITTER, Toronto, Canada. Internatl. Assoc. Ice Cream Mfrs., Prod. and Lab. Council Proc., *2:* 23–25. 1946.

See Abs. 220, Jour. Dairy Sci., *30,* 7: A97. July, 1947.

358. Fruit Purees, Frozen Fruit Desserts and Velva Fruit. E. H. WIEGAND, Food Technology Dept., Oregon State College, Corvallis. Ice Cream Rev., *30,* 12: 52. July, 1947.

Specific directions for the preparation of fruit purees and for their use in the manufacture of frozen fruit desserts and Velva fruit are presented. A simple frozen puree, prepared by the freezing of pulped fruit with or without added sugar, is best adapted for use in ices and sherbets, Velva fruit, and soda fountain flavors. The pectinized puree, which is used in the production of ribbon ice cream, also can be used directly in the manufacture of fruit ice cream without further treatment. Detailed directions are given for the preparation of a pectinized puree, frozen puree desserts, and Velva fruit. W.J.C.

359. Developing Maximum Fruit Flavors in Ice Cream. D. G. SORBER, Albany, Calif. Internatl. Assoc. Ice Cream Mfrs., Prod. and Lab. Council Proc., *2:* 26–40. 1946.

Some fruits are outstanding in flavor and at least 20 kinds are suitable for ice cream manufacture. Full maturity is essential for full flavor development. A disadvantage is that the fruit must be handled very carefully; it is difficult to harvest such fruits as apricots and peaches at one time because they do not ripen uniformly. Soft fruits may be prepared as purees; boysenberries ripen so rapidly that they can only be pureed. Since a portion of the peel aids the flavor of a peach, this fruit must be de-fuzzed before freezing. More fruits are adapted to pureeing than to crushing. Cantaloupes will oxidize rapidly if exposed to air and, therefore, are prepared by the use of a disintegrator which will blanket them from air. Copper and copper alloys and iron must be avoided. Iron and tannin form a gray color in ice cream. Zinc forms poisonous salts. Stainless steel is the best metal to use.

Peaches darken easily with exposure to oxygen and 0.25% ascorbic acid may be added as a preventative, although 0.10 to 0.15% may be sufficient. Number 10 tins to 30-lb. tins are highly desirable for peaches. This fruit may be frozen at −10° F. and stored at 0° F. or below. They should be defrosted at 40° F. and as long as 2 or 3 days allowed for thawing to prevent the definite flavor change taking place at higher temperatures. Apricots are important as frozen fruits, although the variety is not so important. The best are tree ripened and, when crushed, make a good topping.

Avocados may be used to a limited extent, although they darken easily. Fresh bananas may be frozen, and it appears that there will be an abundance of tree-ripened bananas soon. Blackberries vary greatly in quality. Boysenberries, loganberries, Johnsonberries, and Wilsonberries are of the same lineage as the blackberry. Cantaloupes are highly aromatic, can be pureed, and are high in vitamin C, but they must be protected from air or vitamin C will be destroyed. Both sweet and sour cherries may be frozen successfully but not so successfully pureed. Cranberry pulp, 1:1, is good frozen. The Guava contains as high as 1% vitamin C and comes in many varieties. Mangos have a highly aromatic flavor and probably a future as a puree. Nectarines are smooth-skinned peaches more pronounced in flavor, useful with peaches, and one variety is fine for sherbets. The orange is the most common flavor for sherbets. The passion fruit offers great possibilities as an ice cream fruit. Tangerines form an excellent sherbet base. The Santa Rose plum and the red raspberry are well adapted for freezing. P.S.L.

360. Do Winter Ice Cream Supply Shopping Early. C. D. DAHLE. Ice Cream Field, *50,* 1: 40, 43, 44. July, 1947.

Plastic cream, butter, butter oil, and frozen cream are available and now are being purchased for storage. The main point to consider in such purchases is "quality" and the factors affecting quality.

It is recommended that 92 or 93 score butter made from sweet cream pasteurized at 170° F. for 30 min. be stored at 0° F. or lower. Plastic cream is more popular than butter for use in ice cream. Brief mention is made of butter oil and frozen cream. For best results, not over 50% of the fat in ice cream should come from the stored products mentioned here. Care also should be taken to prevent "oiling off"; if it does occur, the use of 0.10% glycerol monostearate or 0.30 to 0.50% dried egg yolk or its equivalent will restore whipping properties of mixes to normal. W.C.C.

361. Should We Lease or Own Retail Stores. ANONYMOUS. Ice Cream Rev., *30,* 12: 114. July, 1947.

When the question of store ownership vs. leasing was put to a group of operators of retail ice cream stores, opinions expressed were divided. In general the operators of a small number of stores who had been able to purchase suitable locations at a reasonable cost favored store ownership. On the other hand, operators of a large number of retail ice cream stores favored leasing rather than owning the property, believing that capital invested in real estate is less productive than when used to provide additional sales outlets for ice cream.

The trend among chain store organizations, as well as among the operators of a large number of retail ice cream stores, is toward leasing rather than ownership of the property. One organization on the West coast reports that they are in the process of selling their stores and taking back long-term leases on these same holdings, thereby releasing capital for further expansion. In the opinion of the operator it is not economically sound for a company with more than 5 stores to put its money into real estate. By turning over all leasing arrangements to a firm specializing in the development of chain store properties, this company has reduced the cost of its leases from an average of 12% to 7% per year. W.J.C.

MILK

362. Pasteurizing Plant Operations in Small Towns. ANONYMOUS. Milk Dealer, *36,* 9: 45, 46, 108–112. June, 1947.

America's new frontier for operators of milk pasteurizing plants, apparently, lies in the nation's rural communities of 800 to 1,500 population where raw milk has reigned supreme and consumer resistance to change consistently has thwarted the venturesome pasteurized milk distributor who sought the necessary business volume to make a pasteurization plant pay. The experiences of the Ambrose Dairy in Cornell, Wisconsin, are related to show the principal problems confronting the small dairy operator in a small community and how such problems may be solved. These problems include: (a) obtaining adequate financing of an improved business venture;

(b) overcoming what is sometimes called "The Psychology of the Small Town"; (c) management; (c) quality control; and (e) product diversification. C.J.B.

363. Clean and Cold. Essentials in the Production of Quality Milk. H. L. Pendleton, Massachusetts Dept. of Agr. Milk Plant Monthly, *36,* 7: 73–74. 1947.

Two essentials for production of high quality milk are cleanliness of production and immediate and maintained refrigeration. Proper sanitation of all utensils with which milk comes in contact is imperative. Storage of rubber inflations in lye solution is recommended. Wetting agents have the property of penetrating the surface of rubber and thus are being used in the cleaning of milker inflations. The brushless age of dairy sanitation rapidly is approaching. G.M.T.

364. Ten Common Causes of Excessive Sediment in Milk. Part 7. Sediment in Rinse Water. C. B. A. Bryant, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36,* 8: 98, 100–101. 1947.

The type of sediment sometimes found in milk may indicate that its source may be coming from the rinse water. Farm tests have shown that the rusty appearing sediment may get in the milk from pipes through the water used for rinsing. G.M.T.

365. Is Mrs. Housewife Continuing to Buy Your Chocolate Flavored Milk? C. F. Bowey, Boweys, Inc. Milk Dealer, *36,* 9: 58–60. June, 1947.

The increase which has taken place in the use of chocolate flavored milk and the factors influencing its quality, price, and merchandising are discussed. C.J.B.

366. What's the Outlook for Paper Containers? J. L. Dolphin, Sealright Co., Inc. Milk Dealer, *36,* 8: 52–58. May, 1947.

The paper shortage largely is due to the fact that many industries that swung over to paper during the war, through necessity, are staying with paper because of convenience. This applies especially to shipping cartons. During the war additional paper mills and machinery for making paper could not be built; it takes 3 years to build a paper mill and to get raw materials, such as pulp, together. Expansion programs currently being executed will produce an additional 3 million tons of paper and paper board per year beginning with 1947, as compared to the capacity in 1945. C.J.B.

367. Quaternary Ammonium Compounds as Preservatives for Milk.

C. K. Johns and H. V. Pritchard, Science Service, Dept. of Agr., Ottawa, Canada. Canad. Pub. Health Jour., *37*, 12: 500–504. Dec., 1946.

The possibility that quaternary compounds might be used as milk preservatives by unscrupulous persons has prompted this study. Four commercial compounds were compared with formaldehyde at their "critical concentration", *i.e.*, the amount in p.p.m. which could just be tasted after holding in fresh raw milk for 18 hr. at 60° F. These concentrations ranged from 70 to 560 p.p.m. for the four products. Formaldehyde could be detected at 25 p.p.m.

To determine the preservative effect at these concentrations, samples of fresh, raw milk were held at 60° F. for 18 hr. and then plated by the standard method. Methylene blue and resazurin reduction times also were determined. The four quaternary compounds were very much less efficient preservatives under these conditions. While each of them exerted a slight bacteriostatic action, these were extremely limited when compared to formaldehyde. The resazurin color numbers after 3 hr. incubation afforded a less variable indication of relative bacteriostasis than was furnished by the plate count and was somewhat more sensitive to small differences than was the methylene blue reduction time. O.R.I.

368. What Can Be Done to Even up Yearly Milk Production.

R. Cowan, Seattle Milk Producers' Assoc., Seattle, Wash. Milk Dealer, *36*, 8: 70–74. May, 1947.

The following plans for evening up yearly production of fluid milk by producing dairymen are discussed: (a) Base and excess (surplus) plan; (b) straight contract with producers for a certain amount of milk each day the year around; and (c) increasing the price in the fall and winter months sufficiently over the spring price to encourage fall and winter production.

The base and excess plan has been in use for many years, and over a period of years it works toward the evening up of production, although not perfectly. The straight contract plan seems more adaptable to markets with large herds. Increasing the price in the fall and winter months would get results but probably would work only where there is a Federal Milk Marketing Order. "An even flow stabilizes the industry for both city milk producer, the factory producer, and it is the best assurance the consumer has to get quality milk at reasonably stabilized prices." C.J.B.

369. Homogenized Milk, Concentrated Milk Products and Other Developments.

C. A. Iverson, Dept. of Dairy Indus., Iowa State College. Milk Dealer, *36*, 8: 49, 50, 84–88. May, 1947.

See Abs. 303, Jour. Dairy Sci., *30*, 9: A129. Sept., 1947.

370. Milk Plant Accounting. F. W. Root, Woodlawn Farm Dairy, Wilkes-Barre, Pa. Milk Dealer, *36,* 8: 46, 112–117. May, 1947.

The relation and contribution of milk plant accounting to efficient management are discussed. Answers must be found to the following questions: (a) Are operations burdened with excessive charges for taxes, insurance, depreciation thus making the initial plant cost too high? (b) Are labor costs too high due to the plant being too large or too small for the volume handled? (c) Does labor maintain a fair standard of productive performance? Are the new employees carefully instructed as to their work and is the supervision adequate to maintain maximum performance? Are there any overmanned operations? (d) Is the plant designed for the most efficient method of handling operations? (e) Is the plant over-equipped, thus causing excess repairs and clean-up time? Are capable engineers available in the event of a breakdown? (f) Are supplies, such as cleaning, bottle caps and hoods, repair parts, and bottles, under inventory control so that amounts used can be checked against actual requirements for the volume handled? (g) Is there a daily accounting for products received, processed, and sold? Do these reports permit the detection of waste? (h) Do steam, refrigeration equipment, and motors throughout the plant have capacities for the most economical operation? Is the maintenance of all equipment on a routine basis which will insure a minimum of interrupted operations?

In conclusion the author states that the accountant can contribute to efficient management by becoming an interpreter instead of a recorder of business information. He should interpret in terms of the future and thus give management an opportunity to prepare for changes; he should become a cooperative finder of facts instead of a seeker of faults. C.J.B.

371. Competing for the Consumer's Dollar. W. Gerber, Whiting Milk Co., Boston, Mass. Milk Dealer, *36,* 8: 152–155. May, 1947.

The nation is drinking 30% more milk than ever before. The opening half of 1946 may be characterized as a highly sheltered seller's market. Price controls remained on milk and other dairy products while other so-called "market basket items" were freed of restrictions. Faced with higher costs, the housekeeper had to use more milk, cream, butter, and cheese. During the war years, too, the public became accustomed to using more dairy products to compensate for the shortages of other basic food staples. Servicemen and women came back from the war drinking more milk than ever before.

The approach to the problem of maintaining and increasing sales is discussed under the following heads: (a) The available market must continue to be educated to the value of milk, the need for increased consumption, and new ways of using milk by means of an all-out advertising campaign embracing all tested media. (b) We must redevelop selling skills. C.J.B.

372. Device for Dispensing from an Inverted Milk Can. L. Bethke. (Assigned to Solar-Sturges Mfg. Co.) U. S. Patent 2,423,679, July 8, 1947 (7 claims). U. S. Patent Office Off. Gaz., *600,* 2: 272. 1947.

A device is described which clamps into the neck of milk cans, forming a base or support for the can when inverted and providing an air inlet and a valve-controlled spout for dispensing the liquid contents. R.W.

PHYSIOLOGY

373. Cobalt Metabolism Studies. III. Excretion and Tissue Distribution of Radioactive Cobalt Administered to Cattle. C. L. Comar and G. K. Davis, Florida Agr. Expt. Sta., Gainesville. Arch. Biochem., *12,* 2: 257. Feb., 1947.

When purebred Jersey steers on an adequate diet were fed labeled cobalt, 80% of the dosage was eliminated in the feces and 0.5% was found in the urine. Relatively high concentrations in the small intestine and intestinal lymph glands indicated the probable route of absorption. The liver was the chief storage organ; an animal sacrificed 5 days after cobalt ingestion contained 0.4% of the dose in the liver.

Labeled cobalt injected into the jugular vein disappeared rapidly from the blood; 65% of the dose was eliminated in the urine and about 30% in the feces. General distribution throughout the tissues occurred; up to 46% of the dose was found in the liver with subsequent excretion *via* the bile. High concentrations were found in the glandular organs. Small amounts of labeled cobalt appeared in the abomasum contents but practically none in the contents of the other stomach compartments. Cobalt administered by injection or fed to pregnant cows was transmitted across the placenta, primarily for storage in the liver of the fetus. The amount reaching the fetus is so small, however, that its significance as a reserve is open to question. H.J.P.

374. A Galactogen from Beef Lung. M. L. Wolfrom, D. I. Weisblat, and J. V. Karabinos, Ohio State Univ., Columbus, Ohio, and O. Keller, Hoffman-LaRoche, Inc., Nutley, N. J. Arch. Biochem., *14,* 1 & 2: 1. July, 1947.

A dextrorotatory galactogen from beef lung was isolated and characterized. The polysaccharide is different from that obtained from the snail *Helix pomatia.* H.J.P.

SANITATION AND CLEANING

375. Developments in Cleaning Compounds for the Dairy Industry. L. H. Minor, J. B. Ford Division, Wyandotte Chemicals Corp. Milk Dealer, *36,* 9: 49–56. June, 1947.

The developments in cleaning compounds for the dairy industry have been correlated closely with the improvement in methods of sanitation for the purpose of safeguarding public health. Forty-six years ago the use of modified soda cleaners for cleaning solutions eliminated soapy off-flavors in milk. Later the pasteurization of milk was accompanied by new cleaning problems which were solved by including silicates and simple phosphates in cleaning compounds. Another advance was made when it was found that the chemicals known as polyphosphates would sequester the mineral substances contained in hard water, thus preventing the formation of the undesirable precipitates. Still later the materials known as surface active or wetting agents were found to assist in obtaining a more rapid penetration of cleaning solutions into milk residues.

The progress made during the past 45 years in the flavor, quality, and sanitary control of milk and its products should be an inspiration for still greater improvements in cleaning, washing, standardizing, and development of germicidal products. C.J.B.

376. Photocolorimetric Method for Determination of Quaternary Ammonium Salts. E. L. COLICHMAN, Turco Products, Inc., Los Angeles, Calif. Indus. and Engin. Chem., Analyt. Ed., *19*, 6: 430–431. June, 1947.

A method is described which consists of forming the quaternary ammonium-bromophenol blue salt in carbonate solution in the presence of an excess but definite critical concentration of dye. The color intensity of the solution is measured directly by photoelectric colorimeter without extracting the colored complex with an organic solvent. The difficulties associated with the use of organic solvents in extraction of color thus are avoided.

B.H.W.

377. Sanitation at the Soda Fountain. C. J. PALMER, Soda Fountain Manufacturers Assoc. Ice Cream Rev., *30*, 12: 60. July, 1947.

The road to a greater prosperity for soda fountain operators is through the operation of a clean, attractive soda fountain. The growing consciousness on the part of the consuming public of possible dangers of infection from spoons, glasses, and similar utensils which have not been properly cleaned and sterilized is causing the public to withdraw its patronage from eating places which are not kept spic and span. Soda fountain operators who do not read the handwriting on the wall and see that their operations are above any possible criticism by a discriminating public will find themselves without customers. It simply is good business to establish voluntarily a reputation for cleanliness and sanitation. Additional patronage and the resultant higher profits which come to the clean, neat, attractive soda foun-

tain far outweigh the extra time, effort, and expense necessary to maintain such an operation. The sanitation program must start with the thinking of management and becomes a reality only when steps are taken to get results.
W.J.C.

378. Methods of Washing, Sterilizing and Drying Cans. V. SCHWARZKOPF, The Lathrop-Paulson Company, Chicago, Ill. Milk Plant Monthly, *36*, 7: 30–32, 34, 46, 53. 1947.

The best method of washing, sterilizing and drying cans is the one which will most completely and economically remove or destroy bacteria present, remove the oil or milk solids, dry the can, protect the tin coating, and keep the can free from water and milk stone. The designer of can washers must know the following with regard to the cleaning compound which is used: (a) its reaction with water hardness, (b) its reaction with milk solids and milk fats, (c) optimum temperatures of use, (d) critical temperatures of use, (e) rinsing characteristics, and (f) effect on surface tension of the rinse water. The conventional method employing alkali detergent and the conservation method employing non-alkaline or acid cleaner now are used for washing, sterilizing and drying cans. The chief difficulties with the conventional method are: (a) continued re-use of the wash water, (b) failure to treat rinse water to prevent scale deposit, (c) high costs because of large volume of hot rinse water, (d) excessive residue contained in the finished cans, and (e) low efficiency in destroying bacteria because of low temperatures. With the conservation method, relatively clean wash water is used continuously; hot water is treated to prevent lime deposit; pumps, pipe lines, and machine are at maximum efficiency since there is no accumulation of lime deposits; cans are more nearly sterile because of high temperature without stone formation; residual alkali is kept at a minimum; and costs of water and steam are cut in half.
G.M.T.

379. Control of Some Insects Affecting Farm Dairying. E. H. FISHER, College of Agriculture, University of Wisconsin, Madison. Milk Dealer, *36*, 9: 140–144. June, 1947.

The following sanitation operations are given for making the control of houseflies, stable flies, and hornflies easier and cheaper: (a) Have well-drained barnyards; flies breed in puddles of decaying straw and similar material. (b) Remove manure frequently (each day if possible) and spread it on the fields. (c) Place straw and hay stacks so that their bases are not continually wet; flies breed in wet straw and hay stacks. (d) Construct privies with a concrete vaunt, a lid on seat, a self-closing door, and screen to cover openings for light and ventilation. (e) Keep garbage cans covered. (f) Use screens on barn and milk house windows. Directions are given for using DDT to control flies and cattle lice.
C.J.B.

380. Bottle Washing Problems. D. H. JACOBSEN, Cherry-Burrell Corp., Chicago, Ill. Milk Dealer, *36,* 9: 154–158. June, 1947.

Bottle washing is discussed under the following headings: soaker washer problems, importance of temperature, bottle washing compounds, scale prevention, cloudy bottles, bottle etching, and alkalis and lubrication in soaker washer. The author concludes that until uniform standards are established, it is well for milk plant operators to follow local standards on caustic concentration and then choose bottle washing compounds which provide the maximum in bottle-cleaning efficiency. The importance of free rinsing and scale-free operation also should be kept in mind. C.J.B.

381. Method of Cleaning Containers. M. E. PARKER AND W. BONEWITZ. (Assigned to P. W. and B. M. Bonewitz.) U. S. Patent 2,424,049, July 15, 1947 (2 claims). U. S. Patent Office Off. Gaz., *600,* 3: 437. 1947.

A method of cleaning containers, such as milk cans, is described which employs an aqueous solution of an organic acid at a temperature in excess of 190° F. to prevent accumulations of calcium and magnesium solids. R.W.

MISCELLANEOUS

382. Glass Block for Exterior Walls. ANONYMOUS. Milk Dealer, *36,* 8: 47, 106. May, 1947.

Glass block is particularly suited to milk processing plant use because it is impervious to moisture and water and is easily cleaned. It diffuses the light into the room, giving uniform illumination, has high insulation value, helps prevent infiltration of dust and dirt, and improves sanitation. Glass block has a "modern look" and is as modern as it looks. C.J.B.

383. Eliminating Fuel Waste. H. D. ELFRETH AND A. MACHARG. Cochrane Corp., Philadelphia, Pa. Milk Dealer, *36,* 9: 92–94. June, 1947.

The service engineer in his routine calls on power plants frequently is astonished at what appears to be a disregard of fuel saving possibilities in the average plant. He frequently notices that: (a) Exhaust steam is being discharged to the atmosphere instead of being used in a feedwater heater to raise the boiler feed temperature. (b) High temperature condensate is being discharged into low pressure receiving tanks vented to atmosphere or into open hotwells. (c) The blowdown from boilers is being discharged directly to waste instead of through a heat-reclaiming system. (d) Even in plants where feedwater heating equipment has been installed, faulty installation or operation occasionally prevents the securing of the saving for which the unit was purchased.

The following is suggested as a check list in determining the savings possible in any particular plant: (a) Maintain a high feedwater temperature. (b) Recover the heat in boiler blowoff water. (c) Return all condensate to the boiler. (d) Reduce moisture content of steam. (e) Check boiler water treatment. (f) Keep on the watch for steam and hot water leaks. (g) Provide insulation or lagging. (h) Practice preventive maintenance.

C.J.B.

384. New Developments in the Field of Refrigeration. W. H. Martin, Dept. of Mechanical Engineering, Oregon State College. Milk Dealer, *36,* 9: 134–138. June, 1947.

Evaporating temperature, discharge pressure, and capacity need to be watched to guard against inefficient refrigeration operation. Rules governing these conditions are given. After discussing the evaporation condenser, moisture in refrigeration systems, and refrigeration system controls, the author presents a table showing comparative data for 1 ton, 200 BTU per min. installation, using Freon 22, ammonia, and Freon 12. C.J.B.

385. Promises and Limitations of Electronics in the Dairy Industry. R. E. Olsen, Taylor Instrument Co., Rochester, N. Y. Milk Indus. Found. Assoc. Bul., *39,* 15: 448–454. May, 1947.

The American Standards Association defines electronics as "that branch of science and technology which relates to the conduction of electricity through gases or in vacuo." The phenomenon was observed by Edison in 1883. In recent years we have had an accelerated development and application of electronic devices.

One of the first applications in the dairy field was the use of the vapor mercury lamp to increase the vitamin D potency of milk. Another application is the use of the so-called germicidal lamps. The electron microscope should be useful in bacteriological studies in the dairy field. Electronic heating has been useful in some other fields, but the pasteurization of milk at present can be accomplished more economically by other means. Many measurements, such as pressure, temperature, liquid level, and time, may be made with electronic devices, but frequently such devices are more complicated or expensive than an alternative method of measurement. Pressure measurement and timing of flow in a STHT pasteurizer are examples. The electronic principle for level controllers has been discarded for a form of power-amplifying transformer because of untimely failure of vacuum tubes for this application.

Temperature may be measured by an electronic device, but mercury thermometers and recording thermometers seem to do the job more simply and inexpensively. E.F.G.

386. What Is Quality? W. Vincent, U. S. Food and Drug Administration, Denver, Col. Natl. Butter and Cheese Jour., *38,* 8 : 47. Aug., 1947.

See Abs. 243, Jour. Dairy Sci., *30,* 7 : A105. July, 1947.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

387. Microscopical Examination of Milk. D. B. SHUTT. Canad. Dairy and Ice Cream Jour., *26,* 6: 32. June, 1947.

A method has been developed whereby the milk smear can be defatted, fixed, and stained in one operation in from 5 to 15 seconds. During this process the background is completely decolorized while the bacteria and cells are stained blue. The method is based upon two formulas using ether, absolute methyl alcohol, methylene blue, and hydrochloric acid. The solution is held in a stoppered bottle and in a refrigerator when not in use. The solution should be filtered before using. H.P.

388. The Effect of Heat-Resistant Reducing Substances on the Dye Reduction Tests Used for Estimating the Hygienic Quality of Pasteurizing Milk. M. A. EDWARDS AND C. S. MORRIS. Starcross, Devon, Eng. Dairy Indus., *12,* 7: 648. July, 1947.

The effect of heat-resistant reducing substances produced by bacteria in milk upon the methylene blue and resazurin tests applied to the milk after pasteurization was studied. Different types of organisms were grown in sterile milk for various periods. The milk was pasteurized and then subjected to the tests immediately and after 24 and 48 hr.

The amount of reducing substances formed appeared to vary with the development of acidity. These substances gradually lose their reducing properties on aging the pasteurized milk. From a practical point of view, the effect of the heat-resistant reducing substances in pasteurized milk probably is quite unimportant, since milk supplies having significant quantities of these substances also would have very high acidity and would be rejected at the receiving platform. D.V.J.

BREEDING

389. Heritability of Amount of Spotting in Holstein-Friesian Cattle. P. BRIQUET AND J. L. LUSH, Iowa State College, Ames. Jour. Hered., *38:* 99–105. 1947.

Three hundred forty-three animals by 24 different sires were studied. The repeatability of visual estimates made at different ages averaged 0.982. The possibility of the slight decrease in amount of white with age as being due to the changing proportion of leg area to body area is suggested. Sex appeared to have no influence on the amount of white.

Heritability estimates were determined by intrasire regressions of offspring on dam and by regression of the amount of white on offspring on average amount of white on parents. With estimates of 99 and 93%, respectively, obtained by these 2 methods, it is concluded that the heritability of individual differences probably is more than 90%. It is suggested that several pairs of genes are required to explain the results obtained. L.O.G.

390. **Cattle Breeding in India.** F. B. Ogilvie, American Delhi Military Area Command. Jour. Hered., *38:* 23–28. 1947.

The work of the Imperial Agricultural Research Institute at New Delhi in developing milk production in the Sahiwal and Tharpakar breeds is considered to make these breeds valuable to southern U.S. because of the climatic similarities of this area to the native homes of these cattle, Punjab and Sind, respectively.

Characteristics of these breeds are absence of dystocia, birth weight of 50 lb. with males slightly heavier than females, milk testing 5% fat, longevity, calving at 28.5 months, and absence (or low incidence) of mastitis. The management practices include a calving interval aimed at 13 months, prenatal (prepartum) milking, calves separated from dams at birth, and 4 × milking. A line breeding program is being followed. Photographs show the get of a bull whose daughters each produced 6,000–7,000 lb. of milk during their first lactation and a string of cows each of which produced over 8,000 lb. of milk.

Foot and mouth disease always is present and an attack may ruin the productive life of a cow. The chief disease problems center around bipolar infection, calf pneumonia, and female sterility. There is no brucellosis in either herd. L.O.G.

391. **Artificial Insemination as a Means of Transmission of Bovine Venereal Trichomoniasis.** D. E. Bartlett, K. G. Teeter, and P. C. Underwood. Amer. Vet. Med. Assoc. Jour., *111,* 845: 114–116. Aug., 1947.

The possibilities of transmission of Trichomoniasis from infected bulls to uninfected cows by means of artificial insemination is discussed. Of 9 uninfected females inseminated with fresh, undiluted semen collected from an infected bull by means of an artificial vagina, 6 became infected as a result of 7 inseminations; 3 did not contract the disease after 6 inseminations. T.M.L.

392. **Duration of Sperm Fertility in the Ewe.** W. W. Green. Amer. Jour. Vet. Res., *8,* 28: 299–301. July, 1947.

Fifty-five grade Shropshire ewes and 2 highly fertile grade Shropshire rams were used in this experiment. The ewes were bred from 0 to 54 hr. after the end of estrus. The ewes that did not return in heat were slaughtered and the fetuses recovered. The ewes returning to heat were counted as non-pregnant.

The data reveal that sperm may retain the ability to fertilize an ovum after approximately 2 days' residence in the reproductive tract of the ewe.
T.M.L.

BUTTER

393. The Titratable Acidities of Cream and the pH of Commercial Butter. A. H. WHITE. Canad. Dairy and Ice Cream Jour., *26*, 6: 21. June, 1947.

The neutralizing data of 92 commercial and 75 semi-commercial churnings showed that the pH of butter can be maintained between the recommended range of 6.7 to 7.2 by accurate neutralizing procedures. From 75 to 82.5% of the churnings of 3 creameries analyzed over a period of a year were within this range. For cream with an initial acidity of 0.25–0.3%, the acidity should be reduced to 0.11–0.12%. In general, for each 0.1% increase in the initial titratable acidity, the desired acidity after neutralization should be increased by 0.01%. Variations in the pH of butter from any one plant were due to inaccuracies in determining the initial acidity of the cream, in estimating the weight of cream, and in calculating the amount of neutralizer to use.
H.P.

394. Standardizing the Composition of Butter. R. W. BROWN. Canad. Dairy and Ice Cream Jour., *26*, 6: 26. June, 1947.

A simple and accurate method of determining the amount of water to add to a churning of butter in order to standardize its composition is to make use of the "Pearson Square" method used in standardizing milk and cream.
H.P.

395. Butter Boxes. R. W. BROWN AND T. L. FORSTER. Canad. Dairy and Ice Cream Jour., *26*, 5: 31. May, 1947.

Examination and analysis of 7 boxes of Australian and New Zealand butter revealed that much paper fiber is used in the fabrication of the boxes. The all-fiber boxes showed up the best from the standpoint of flavor and sediment in the butter surfaces.
H.P.

396. The Power Butter Printer. J. C. PALMER AND P. J. BOGAERTS. Canad. Dairy and Ice Cream Jour., *26*, 7: 28. July, 1947.

In printing butter with a power printer it is important to have the butter evenly tempered throughout. The butter should be well worked and show no free moisture. If printed too cold, some loss in moisture will result, accompanied by off color in the print. Streakiness can be overcome by: (a) not allowing the machine to operate unnecessarily with butter in the hopper; (b) feeding the machine evenly with even pressure; (c) printing butter between 37 and 47° F.; (d) in the case of twin auger machines, setting the edges of the spiral so that they meet when they turn; and (e) holding butter in the refrigerator for at least 24 hr. before printing. H.P.

397. **Paper Board Flavor on Print Butter.** D. B. SHUTT. Canad. Dairy and Ice Cream Jour., *26,* 7: 27. July, 1947.

Butter prints packed in unlined cartons developed a very disagreeable flavor, which was variously described as disinfectant, iodine, and even as chloride of lime. Corrugated paper cartons that are to be used for holding print butter always should be lined with waxed or other waterproof paper. H.P.

CHEESE

398. **The Pink Test.** I. HLYNKA, E. G. HOOD, AND C. A. GIBSON. Canad. Dairy and Ice Cream Jour., *26,* 5: 25. May, 1947.

A new test is proposed for determining setting time in Cheddar cheesemaking. It depends upon the reduction of resazurin to its pink end-point and is called the "pink test". One milliliter of standard resazurin solution is pipetted into a test tube to which a 10-ml. quantity of milk from a well-stirred vat is added. The tube is held at 86° F. and examined at the end of 5 minutes or until the original color is reduced to pink. The vat is ready to set when this color change takes place in 5 minutes. Cheesemaking experiments using starter at different levels were carried out in order to test the performance of the pink test. The pink test is based on a sound principle; it measures the rate of lactic acid development in the vat by measuring its rate over a short interval in cheese milk. H.P.

399. **Cheese Making from Milk Pasteurized at High Temperatures.** E. ANDERSEN, Danish Government Experimental Dairy Station, Hillerød, Denmark. Nordisk Mejeri-Tidsskrift,* *12,* 9: 177–179. 1946.

Cheesemaking from milk pasteurized at high temperatures was tried for the first time more than 50 years ago in Denmark, but without success. Up to 1946 the method still proved unsuccessful.

The 6th annual publication from the Danish Government Experimental Dairy Station deals with the problem in connection with testing of a "Spirala" pasteurizer for cheese milk. "Danish Swiss cheese" has been made from milk pasteurized at 80° C. (176° F.) and 90° C. (194° F.). By changing the cheesemaking procedure for cheese made from milk pasteurized at high temperatures, cheese of a fair quality could be made as compared with cheese made from milk pasteurized at 70° C., flash-pasteurization. Holding times of 21 and 65 seconds were used. The higher temperature and the longer holding time resulted in the greater yield. Yield increases ranged from 4% at 80° C. held for 65 seconds up to 10% at 90° C. held for 65 seconds. The latter cheese was likely to be sour. Cheese made from milk pasteurized at high temperatures ripens very slowly. It is doubtful if the increased yield compensates for cost of pasteurization and reduction in quality.

Heating of milk to 80° C. or above causes changes in the colloidal calcium salts and coagulates the albumin. As a result of the changes in colloidal calcium salts, the coagulation of the milk by rennet is reduced considerably and the curd formed is abnormal. The precipitation of the albumin makes it difficult to get the whey out of the curd, and the cheese develops a short and brittle consistency so the grains will not stick together. The cheese will come from the press with a greater whey content than normal and consequently a higher lactose content. The pH of such cheese will have a tendency to be too low, the cheese becoming sour. The precipitation of the albumin is responsible for the greater yield, as the curd binds more water and the albumin goes into the cheese instead of into the whey.

Coagulation of the milk can be helped considerably by adding calcium chloride to the milk. Up to 30–50 g. per 100 kg. (220 lb.) of milk can be used. If the temperature is raised 2–3° C. (normal setting temperature, 86° F.), an almost normal curd can be obtained. Sour cheese can be prevented by using less starter, but the best way is to raise the cooking temperature and increase the amount of water used for heating purposes.

"Danish Swiss cheese", Port Salut, and other kinds of cheese of the "Tilsiter" type can be made from milk pasteurized at high temperatures, but the method is not good for Gorgonzola, Blue cheese, and Swiss. T.K.

400. Further Studies on the Measurement and Control of Physical Factors in Cheese Making.

MARGARET BARON. Dairy Indus., *12*, 6: 548. June, 1947.

Two studies, one on Cheddar cheese and the other on long-keeping Cheshire cheese, were conducted. The physical properties of the cheese at different points of manufacture were correlated with the properties of the finished cheese. Bowl, ball compressor, and borer techniques were employed.

The bowl readings taken on the curd just before cutting appear to be closely related to the properties of the finished cheese. Numerous correlations were run involving such factors as milling acidity, milling time, solids-not-fat, and butterfat content. Tables showing the correlation coefficients developed from the data are presented. D.V.J.

401. Packaging Natural Cheddar Cheese. O. R. IRVINE. Canad. Dairy and Ice Cream Jour., *26*, 6: 27. June, 1947.

The wrapping of prints of cheese in transparent plastic film is a new development. Cheese lends itself remarkably well to wrapping in transparent films. In order to be effective, the package should protect the product's flavor, prevent mold growth, and have a fairly long shelf life. Among other desirable features in the package are low cost and ability to withstand abuse at storage temperatures. The package also should carry colorful, clear, and readily seen branding. The method of packaging in films is called "pressure packing". The cheeses, usually in 20-lb. squares, are cured until the desired state of maturity is reached, after which the blocks are cut out into small prints and made ready for retail sale. H.P.

402. Packaging Cheese Curd. L. H. JONES. (Assigned to Wingfoot Corp.) U. S. Patent 2,424,693, July 29, 1947 (3 claims). U. S. Patent Office Off. Gaz., *600*, 5: 746. 1947.

After removing the press cloth from cheddar-type cheese, the curd is placed in a bag made of thin, limp, moisture-proof and oil-resistant material and placed in a rack designed in such a manner that the cheese may be cured in the container under pressure in an air-free condition. R.W.

CHEMISTRY

403. Pennsylvania Method for Butterfat. J. R. EASTMAN AND G. R. MAYBEE. Canad. Dairy and Ice Cream Jour., *26*, 5: 64. May, 1947.

With raw milk samples testing 4.1–4.4% butterfat by the standard Babcock test, the addition of n-butyl alcohol to the test bottle increased the test to 0.13, 0.20, and 0.26% by the addition of 1.5, 2, and 3 ml. of n-butyl alcohol, respectively. Two milliliters of ammonium hydroxide as specified in the Pennsylvania modification of the Babcock test had no effect on the test. Results confirm that the decrease in reading due to glymol addition helps to offset the increase due to the addition of n-butyl alcohol in the Pennsylvania modification of the Babcock test. The combined effect of glymol and 3 ml. of n-butyl alcohol will cause the results to be about 0.20% higher than those by the Babcock test on raw milk samples of about 4% fat content. H.P.

404. Relationship of Acetone Bodies to "Cowy" Flavor in Milk. D. V. JOSEPHSON AND D. G. KEENEY, Dept. of Dairy Technology, Ohio State University. Milk Dealer, *36*, 10: 40–42. July, 1947.

Experiments are reported showing that much of the "cowy" or "barny" flavored milk received at milk plants during the winter months is due primarily to chemical substances, *i.e.*, acetone bodies, which are secreted in the milk of cows suffering from ketosis or acetonemia. The disease is common in well-managed and high-producing herds and seldom is encountered when cows are on pasture. The data reported also suggest that some of the "feed" flavors observed in mixed milks also may be due to low concentrations (less than 25 p.p.m.) of acetone bodies. C.J.B.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

405. Titratable Acidity of Milk Powders. A. H. WHITE. Canad. Dairy and Ice Cream Jour., *26*, 8: 27. Aug., 1947.

Acidity tests were made on a number of samples of different types of dried milks to determine the probable causes of variations obtained in the acidity tests of the same sample by different laboratories. Variations in the acidity test were found between the top and bottom portions of reconstituted roller-dried milks. The variations were 0.025 to 0.07% after being held for 15 min. or less, and 0.03 to 0.105% after being held for 1 hr. or longer. The top and middle portions of the reconstituted roller-dried milks decreased in acidity as the time of holding increased. Similar tests on spray-dried skim and whole milks showed no variations in acidity even after being held for over 1 hr. Thorough agitation and mixing of reconstituted roller-dried powders is essential if agreement in acidity tests of the same sample is to be obtained in the same laboratory or different laboratories. H.P.

406. How to Make Yoghurt. L. A. BURKEY, Division of Dairy Research Laboratories, U. S. Dept. of Agr. Milk Dealer, *36*, 10: 43, 44, 114. July, 1947.

Complete information on yoghurt manufacture is reported. Directions for handling the culture, steps in carrying mother cultures, preparation of the bulk starter, and the materials and equipment needed for the preparation of yoghurt are given. C.J.B.

DISEASES

407. A Clue to the Cause of Milk Fever in the Metabolism of the Springing Cow. A. H. CRAIGE, JR. Amer. Jour. Vet. Res., *8*, 28: 247–256. July, 1947.

A study of the causes and treatment of milk fever was conducted on a group of low producing cows not particularly susceptible to the ailment. Clinical symptoms of milk fever and also death were induced by artificial alkalization produced by oral and intravenous administration of sodium carbonate to non-parturient cows. Oral administration of Chlor-Ethamine induces rises in blood calcium and phosphorus in parturient cows. In nonparturient cows Chlor-Ethamine has little or no effect.

A tentative explanation for milk fever is based on the sudden demand for active withdrawal of various electrolytes from the blood stream into the colostrum and suppression of efficient calcium mobilization in the body as a result of a developing alkalosis. Further conjecture suggests that acetonemia or ketosis may be a result of compensatory mechanisms, which in some cases completely counteract the physiologic alkalizing process. T.M.L.

408. Sensitivity to Penicillin of Microorganisms Associated with Bovine Mastitis. J. O. Heishman. Amer. Jour. Vet. Res., *8*, 28: 257–260. July, 1947.

Results of the examination for sensitivity to the action of penicillin of 150 cultures of bacteria isolated from the milk of cows having varying degrees of mastitis are reported. The inhibition range for *Streptococcus agalactiae* and *Streptococcus uberis* varied between 0.031 and 0.125 units of penicillin per cc. of medium. The coliform rods were not affected by 2 units of penicillin per cc. of medium. T.M.L.

409. Treatment of Bovine Anthrax with Penicillin. C. W. Riggs and A. C. Tew. Amer. Vet. Med. Assoc. Jour., *111*, 844: 44–46. July, 1947.

Treatment of anthrax by serum alone, serum and penicillin, and penicillin alone are compared. The treatment of 93 cases indicates that penicillin is an effective means of combating the infection and that it is as effective given alone as it is when given in combination with serum. T.M.L.

410. Further Observations on the Virus Diarrhea (New Transmissible Disease) of Cattle. P. Olafson and C. G. Richard. Cornell Vet., *37*, 2: 104–107. April, 1947.

The relaton of rinderpest to virus diarrhea is discussed. The antirinderpest serum was used on a small number of calves to combat virus diarrhea without any success. The virus disease probably is independent of rinderpest, although some of the symptoms are very similar. T.M.L.

411. Staphylococcic Abortion in Cattle. W. D. Pounden and W. E. Krauss. Amer. Vet. Med. Assoc. Jour., *111*, 844: 35–37. July, 1947.

Two cases of abortion in late pregnancy in dairy cows are described. Both cows conceived to artificial insemination and, at the time of abortion, copius uterine pus exudates were present. Gram-positive cocci were observed in large numbers in all smears. T.M.L.

412. Blood Transfusions in Bovine Anaplasmosis. J. C. Lotze. Amer. Jour. Vet. Res., *8*, 28: 284–289. July, 1947.

Five cases of experimentally produced bovine anaplasmosis were treated by blood transfusion. It is concluded that in certain cases, blood transfusions are indicated due to the inability of the host to regenerate red blood cells rapidly enough to overcome quickly the severe anemia caused by the infection. To be effective, blood must be given in large quantities. T.M.L.

413. Use of Sodium Sulfonamides as Single Injection Specific Treatment in Foot Rot. C. R. Forman, J. E. Burch, C. E. Dee, L. Kelley, J. E. Mouw, M. B. Teigland, and J. H. Yarborough. Amer. Vet. Med. Assoc. Jour., *111*, 846: 208–214. Sept., 1947.

Results from treatment of 1,500 cases of foot rot with sulfonamides are presented. The intravenous injection of sodium sulfapyridine is the method of choice in treatment of *Actinomyces necrophorus* infections in cows. Treatment is simple and specific. T.M.L.

FEEDS AND FEEDING

414. A Chick Growth Factor in Cow Manure. V. Relation to Quantity and Quality of Soybean Oil Meal in the Diet. M. Rubin and H. R. Bird, Bureau of Animal Industry, Agr. Research Center, Beltsville, Md. Jour. Nutr., *34*, 2: 233. Aug. 11, 1947.

A diet of 70% soybean oil meal caused an inhibition of growth and increased mortality in chicks. These effects were not counteracted by the addition of methionine but were counteracted by the addition of cow manure or cow manure extracts. The growth factor of cow manure improved diets containing heated soybean oil meal more than those containing unheated meal. R.K.W.

FOOD VALUE OF DAIRY PRODUCTS

415. Comparative Nutritive Value of Casein and Lactalbumen for Man. A. J. Mueller and W. M. Cox, Jr., Mead Johnson and Co., Evansville, Ind. Jour. Nutr., *34*, 3: 285. Sept. 10, 1947.

Casein and lactalbumen were fed at an intake of 2% of the calories to 4 adults following 12-day depletion periods. Four-day periods of supplementation with one protein were followed by 4 days of depletion and then 4 days of supplementation with the alternate protein. Each protein was fed for two such 4-day periods. Nitrogen balances and biological values indicate that casein and lactalbumen are equally effective in maintaining nitrogen balances in man. R.K.W.

416. The Sparing Action of Protein on the Pantothenic Acid Requirement of the Rat. II. Urinary and Fecal Excretion of Pantothenic Acid. Marjorie M. Nelson, F. Van Nouhuys, and H. M. Evans, Institute of Experimental Biology, Univ. of Calif., Berkeley. Jour. Nutr., *34,* 2: 189. Aug. 11, 1947.

It had been shown that high casein diets had a sparing action on the pantothenic acid requirement of the rat. In these studies the protein contents of diets were varied by using diets containing from 24 to 64% casein. The data indicate that the sparing action for pantothenic acid of the high casein diet is real and not due to decreased urinary excretion or increased intestinal synthesis. R.K.W.

417. The Growth-Promoting Action of Cellulose in Purified Diets for Chicks. F. Davis and G. M. Briggs, Univ. of Md. Jour. Nutr., *34,* 3: 295. Sept. 10, 1947.

Supplementation of a simple purified diet, containing casein, with cellulose at levels of 5, 10, and 15% increased gain and feed efficiency for young chicks up to 4 weeks of age. Larger amounts of cellulose decreased growth. R.K.W.

ICE CREAM

418. Fruits and Their Importance in Ice Cream. I. S. Brightman, Fruit and Syrup Manufacturers Association. Ice Cream Rev., *31,* 1: 69. Aug., 1947.

Because of the eye and taste appeal which they give to frozen desserts, fruits have been an important factor in building ice cream sales to their present level. Today with more than 300 million lb. of fresh and cold packed fruits available, an adequate supply for use in ice cream is assured. Today fruits are available which are being processed to conform with the Food, Drug and Cosmetic Act. This means that the ice cream manufacturer will be supplied with fruits honestly packed, and which are free from insect infestation, mold, bacteria, or other foreign material. W.J.C.

419. Ice Cream Disher. A. Gordon and S. G. Mozolewski. U. S. Patent 2,427,095, Sept 9, 1947 (5 claims). U. S. Patent Office Off. Gaz., *602,* 2: 279. 1947.

A scoop type disher is described with a scraper blade, operated by a pivotally mounted actuating device. R.W.

420. Packaged Ice Cream. F. Yoars. Canad. Dairy and Ice Cream Jour., *26,* 8: 30. Aug., 1947.

Quick freezing and quick hardening of ice cream can be accomplished within a matter of 20–30 min. by use of the wind tunnel. Quality thus can be improved more statisfactorily than by adding more butterfat, which adds cost to the product. Pre-molded portions eliminate bulk ice cream, which, in turn, eliminates dipping and reduces supervision and dispensing difficulties. H.P.

MILK

421. Farm Milk Cooling Requirements. Anonymous. Milk Dealer, *36*, 10: 116–124. July, 1947.

Results of a survey show that there is a trend toward mechanical cooling of milk on the farm. In the complete survey made of 55 cities in 34 states, only 5 cities reported mechanical cooling on the farm as a present positive requirement. Numerous other cities reported, however, that for all practical purposes such a requirement was in effect because it is impractical to meet temperature requirements unless the producer is equipped with mechanical refrigeration. This is shown by the reports from 52 cities which gave specific information on temperature requirements for milk upon delivery at the plant receiving platform. Of these, 10 reported requirements of 50°, 3 of 55°, 15 of 60°, 4 of 65° and 20 of 70° F. C.J.B.

422. Milk Cooler. G. R. Duncan. U. S. Patent 2,425,519, August 12, 1947 (10 claims). U. S. Patent Office Off. Gaz., *601*, 2: 266. 1947.

In a cabinet for cooling milk in cans, a cooling liquid is sprayed around the necks of the cans and down the sides to collect in a sump beneath the cans, to be cooled again and recirculated. R.W.

423. Milk Machine. E. B. Thompson and F. A. Gessler. (Assigned to Globe Milker, Inc.) U. S. Patent 2,427,312, Sept. 9, 1947 (16 claims). U. S. Patent Office Off. Gaz., *602*, 2: 334. 1947.

This apparatus consists of a self contained receptacle for receiving the milk through a connection from a teatcup, a pair of air chambers operating at subatmospheric pressure, an electrical motor for operating the pump, and an air distributing device. R.W.

424. Frozen Milk with Special Reference to Protein Flocculation. F. J. Doan and F. G. Warren. Canad. Dairy and Ice Cream Jour., *26*, 7: 60. July, 1947.

Milk can be stored in the frozen condition either in the fluid or concentrated state for considerable periods of time. High heat treatment at time of pasteurization delays the onset of oxidation and deterioration of flavor of frozen milk. Stability of frozen milk is decreased by removal of water, by high heat treatment, by high storage temperature, and by long periods of storage. H.P.

425. **Homogenized Milk, Concentrated Milk Products and Other Developments.** C. A. IVERSON. Canad. Dairy and Ice Cream Jour., *26*, 5: 37. May, 1947.

See Abs. 303, Jour. Dairy Sci., *30*, 9: A129. Sept., 1947.

PHYSIOLOGY

426. **Physiologic Reactions to Intravenous Calcium Injections in the Cow.** A. H. CRAIGE, JR. Amer. Jour Vet. Res., *8*, 28: 260–267. July, 1947.

A series of 16 experiments were conducted by intravenous injection of 500 cc. of calcium gluconate in non-parturient cows. A sudden increase in blood calcium and phosphorus results after intravenous injection, followed by a progressive decline until 24 hr. later, when the normal values are approached. T.M.L.

SANITATION AND CLEANSING

427. **Sanitary Standards for Dairy Equipment.** E. H. PARFITT, Chairman, Sanitary Standards Subcommittee, Dairy Industry Committee. Milk Dealer, *36*, 10: 136–142. July, 1947.

A complete description of the purpose, organization, accomplishments, and of the work in progress of the Sanitary Standards Subcommittee is reported. In conclusion, the author states that: "The dairy industry is now well organized through all its branches to do an outstanding job in arriving at sanitary standards for dairy equipment. The final answer as to the success of this undertaking will depend upon: (1) The backing that is given to these standards by the individual milk sanitarian, and his willingness to go along with these standards rather than attempt to modify them according to his individual thinking. (2) The publicity that is given to these standards so that buyers of equipment for which standards have been developed will specify the accepted 3A sanitary standards. (3) The degree of support that is given by the regulatory groups in seeing to it that fabricators whose equipment does not meet the sanitary standards is not designated or sold as meeting the 3A standard." C.J.B.

428. **The Use of a Quaternary Ammonium Compound as a Supplement to Heat in the Rinse in Mechanical Dishwashing.** W. L. MALLMANN AND L. ZARKOWSKI, Division of Bacteriology and Public Health, Mich. Engin. Expt. Sta., Lansing. Jour. Milk and Food Technol., *10*, 4: 206–213. July–Aug., 1947.

Washing and sanitizing dishes in a mechanical dishwasher in which the temperature of the rinse water is maintained at 170° F. reduces the health hazards to a safe margin. However, lunch counters and soda fountains frequently resort to hand washing, with the temperature of the rinse water too low to be effective in the destruction of pathogenic organisms. Any attempt to supplement hot water rinsing by using sanitizing agents is highly desirable.

The authors conclude that quaternary ammonium compounds in concentrations of 1–6,400 to 10,000 at a temperature of 120° F. will destroy both Gram-positive and Gram-negative bacteria in 5 to 10 seconds in the absence of organic matter. This procedure is effective in sanitizing machine-washed dishes where high-temperature water is not available or is impractical to use. H.H.W.

429. Inconsistencies in Testing and Reporting the Strength of Alkaline Detergent Solutions. C. W. ENGLAND. C. Y. Stephens Dairy and Poultry Industries, Washington, D. C. Milk Dealer, *36*, 10: 49–56. July, 1947.

The literature on testing and reporting the strength of alkaline detergent solution is reviewed. This review serves to indicate the duplicity of terms or designations and the confusion relative to reporting results of tests. There is wide diversity in the amount of sample used and the method of analysis. A system of reporting the results of the analysis of alkaline detergent solutions is proposed. C.J.B.

430. D.D.T. and Dairy Sanitation. C. F. GERLACH, Michigan Chemical Corp. Milk Dealer, *36*, 10: 156–160. July, 1947.

Insects may affect dairy products directly by contact and infestation and indirectly by contamination of containers, carrying contaminating organisms, and reducing production and quality. DDT has proved not only an aid to sanitation but a profitable investment. Increased milk production and quality have returned profits many times over and above the cost of the DDT program. Directions are given for controlling flies, lice, ticks, silverfish, roaches, ants, and fleas on dairy farms. Directions also are given for insect control in dairy plants. C.J.B.

431. Elimination of Multiple Inspections and Appraisal for Various Plants and Localities. W. A. WENTWORTH, The Borden Co., New York, N. Y. Milk Indus. Found. Assoc. Bul., *39*, 17: 491–500. June 25, 1947.

Faults in the present milk inspection system and recent experience have suggested the following conclusions: (a) The quality of a milk supply cannot be judged by the ordinances governing its production and processing because of frequently ineffective enforcement. (b) There are extreme variations in milk quality between areas which have identical ordinances. (c) Re-evaluation and modernization of our milk ordinances should be of immediate concern to the industry and public health officials. The problems of use of milk inspection to raise trade barriers and the burdens of duplicate and conflicting regulations are discussed. The author suggests an effort in the field of city milk supply on the lines of the work of the Dairy Products Improvement Committee for sweet cream. Another industry-wide effort is the work of the Sanitary Standards Committee of the Dairy Industry Committee, which, in conjunction with the International Association of Milk Sanitarians, is developing appropriate standards for milk plant equipment. E.F.G.

MISCELLANEOUS

432. Agricultural Marketing Agreements—A Review. W. C. Welden, H. C. Hood and Sons, Inc., Boston, Mass. Milk Indus. Found. Assoc. Bul., *39*, 17: 483–490. June 25, 1947.

The first license issued Aug. 1, 1933, covered the Chicago Milk Market. Since the inauguration of this practice under the Agricultural Adjustment Act of May, 1933, there have been close to 100 licenses or orders issued by the Secretary.

During the war operation of marketing orders tended to cause more rapid price adjustment under OPA, and these markets were more adequately supplied with milk than other areas. A trend in marketing order techniques is to price milk by formula, which simplifies revision of prices both up and down.

Prospective problems of federal control involve two angles: (a) Can a more balanced approach be developed to point orders toward industry and the public welfare as well as producer welfare? (b) Can the orders be made really effective in promoting steady and adequate milk supplies at reasonable and stable prices? E.F.G.

433. Functions and Responsibilities of Leadership in the Dairy Plant. J. H. Erb. Canad. Dairy and Ice Cream Jour., *26*, 7: 48. July, 1947.

Competent supervision should be able to control quality so that it is uniformly good day after day. In order to work out a quality problem, the following steps should be taken: (a) Analyze the problem, (b) plan the solution, (c) sell top management on changes, (d) train subordinates, and (e) check back. The maintenance of equipment requires precedence in the time and energy of plant supervision. Plant supervision should play a role in cost control. Ingredients and finished products should be accounted for and handled like money. The proper type of leader will have to be developed by management by better selection of leaders, providing a training program for developing greater leadership skills, reducing unreasonable load so there is more time to study and work on "problem" areas, and bringing middle supervision closer to management. To qualify for the job of being a leader one must have training, not only past training and experience but training while on the job. H.P.

434. A Few Basic Considerations in Dairy Economics. D. B. DELOACH. Canad. Dairy and Ice Cream Jour., *26*, 7: 43. July, 1947.

The milk industry of today is the result of the ability of the industry to utilize fully the scientific background of the 19th and early part of the 20th centuries. The milk industry has the unusual advantage of almost universal acceptance of its products as a basic food for children and adults. It has received favorable attention in the health and educational programs of our public school system and has become an item essential to the cookery techniques of every modern homemaker. The components of efficient plant operations will have to center around the following: (a) procurement costs and plant location, (b) product utilization and market prices, (c) plant volume, (d) transportation to market, and (e) packaging. H.P.

435. Problems in the Design and Layouts of Dairy Plants. R. T. SOUTHMAYD. Canad. Dairy and Ice Cream Jour., *26*, 8: 62. Aug., 1947.

The market which a plant will serve and the amount of business it may be expected to do should be used in determining its most efficient and flexible layout. The lot size is very important as it determines the size of the plant, free and easy handling of traffic, good appearance, and friendly relations with neighbors. Three general problems of the plant proper are size and location of the various work spaces, type and location of processing equipment, and type, size, and location of mechanical equipment. All the individual problems must be blended into a co-ordinated unit that will utilize human effort efficiently. H.P.

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

436. Feeds of the World, Their Digestibility and Composition. B. H. SCHNEIDER. 299 pp. $3.00. West Virginia University, Morgantown. 1947.

This is a comprehensive summary of available information on the composition of feeding stuffs and their digestibility by the five most common species of mammalian farm animals, namely: cattle, sheep, goats, swine, and horses. The data presented resulted from a thorough analysis of American and foreign literature involving approximately 11,000 original digestion experiments conducted during the past 80 years.

The book is divided into four sections. The first two are bibliographical, listing the authors whose research data have been included in this publication and the feeding stuffs and species of animals studied by each. Section I is arranged alphabetically by authors and section II is a cross-index of the first, arranged alphabetically by feeding stuffs. Section III lists the common and scientific names of plant and of animal sources of feeding stuffs. The final section consists of a tabulation of the composition and digestibility of the various feeds. With the exception of the table including data from investigations with sheep and with goats, the results from digestion trials are presented separately according to species of animal. Data tabulated include digestible nutrients and composition of feeding stuffs as offered to animals, digestion coefficients, and digestible nutrients and composition of feeds on a moisture-free basis.

This compilation has required a tremendous amount of time and effort on the part of the author. Since this book is the first of its kind to be published, it should be of great service to all who are interested in livestock nutrition or composition of feeding stuffs. N.L.J.

437. Notas sobre a bioquimica do leite (Notes on the biochemistry of milk). O. BALLARIN. Meier & Blumer, Rio de Janeiro, Brazil. 150 pp. 1947.

This book was written in Portuguese with the purpose of surveying the recent knowledge of the biochemistry of milk and facilitating the understanding of the various phenomena concerning this product. It is divided into four major parts. In the first part, milk is defined and its organoleptic characteristics and physical, chemical, and biological properties are discussed. The second part is devoted to the study of the chemical composition of milk; this is treated with comparatively greater detail than is employed in the other portions of the book. The action of the various chemical, physical, and biological agents upon milk is discussed in the third part. The presentation of a number of charts shortens and facilitates this discussion. The metabolism of milk is discussed in the fourth section. A diagram summarizes this portion of the book. The appendix gives some very general information concerning the various milk products.

The book is written in a very definite and organized sequence. Charts, diagrams, and illustrations are presented generously to facilitate the understanding of the subject. Throughout the book, the different facts discussed are related to the various problems encountered in the practical dairy industry work. However, little detail is given in regard to the actual commercial processing of milk, and the reader is expected to resort to other books for this kind of information. Most of the references are to condensed, evaporated, and powdered milk, with few references to cheese, ice cream, and butter.

Frequent citations to the original publications, including many which have appeared in recent years, are made throughout the book. Failure to arrange the bibliographical citations either alphabetically or chronologically and to adopt a uniform system of citation in the text of the book reduces the usefulness of this portion of the work. R.E.M.

BACTERIOLOGY

438. Proposed Changes in Incubation Temperatures for Standard Agar Plate Counts (Abs.). J. D. BREW, Univ. of Tennessee. Jour. Bact., *53*, 3: 372. March, 1947.

Forty years ago agar plates were incubated 5 days at 20° C. plus 2 days at 37° C. on the assumption that most bacteria, including pathogens, would grow. Because the 7-day incubation was impractical, 37° C. for 48 hr. became accepted. Lately it has been observed that there are greater variations in plates incubated at 37° C. than at lower temperatures. The maximum growth range for some bacteria is close to or above 37° C. Intra- and inter-incubator temperatures vary more at 37° C. than at lower temperatures, some incubators showing zones as high as 45° C. It is proposed to lower incubation temperature to 32° C. for 48 hr.

439. A Study of Variability in Duplicate Standard Plate Counts as Applied to Milk (Abs.). J. L. COURTNEY, Oak Ridge Dept. of Health, Oak Ridge, Tenn. Jour. Bact., *53*, 3: 372. March, 1947.

Of 299 duplicate counts made on raw milk, 278 varied less than 50%, 18 between 50 and 100%, and 3 over 100%, the highest being 191%. The average variation was less than 20%. The author deplores the attitude that there is no point in exercising care with the technique because "the error inherent in the method is greater than the error which will be introduced''. Rather, human error accounts for much of the so-called error of the method.

D.P.G.

440. A Factor Toxic to *Brucella abortus* in Some Lots of Tryptose (Abs.). V. T. SCHUHARDT AND L. J. RODE, The Brucellosis Research Project of the Clayton Foundation and the Univ. of Texas, Austin. Jour. Bact., *54,* 2: 282. Aug., 1947.

Three lots of Difco tryptose specifically suppressed growth of 5 strains of *Brucella abortus.* The factor was not active against strains of *B. melitensis, B. suis,* or 6 other bacterial species. The factor in 2% tryptose was brucellacidal in 48 hr. against 400 to 500 organisms per ml. The factor is neutralized by blood, serum, Difco agar, and aqueous extracts of a number of plant and animal tissues. D.P.G.

441. A Superior Culture Medium for the Enumeration and Differentiation of Coliforms (Abs.). G. H. CHAPMAN, Clinical Research Laboratory, N. Y. Jour. Bact., *53,* 4: 504. April, 1947.

The medium has the composition: water, 1,000 ml.; Difco yeast extract, 3.0 g.; Proteose no. 3 peptone, 5.0 g.; lactose, 10.0 g.; agar, 15.0 g. Adjust to pH 6.9 and add 0.1 ml. tergitol-7 and 2.5 ml. of 1% bromthymol blue. Incubate 20 hr. at 37° C. *Escherichia* produces yellow colonies surrounded by yellow zones. *Aerobacter* produces greenish-yellow "gum drop" colonies, larger than *Escherichia* colonies and usually surrounded by yellow zones. Colonies of paracoli and other organisms not fermenting lactose usually are surrounded by blue zones. Some strains of *Neisseria catarrhalis* grow but produce minute, rough, blue colonies with blue zones. No other bacteria have been observed. *Proteus spp.* have less tendency to spread. There is no inhibition of coliforms, permitting recovery from minute inocula. Counts are about 30% higher than on other media. D.P.G.

442. The Relationship between *Lactobacillus acidophilus* (Moro) Holland and *Lactobacillus casei* (Orla-Jensen) Holland. C. S. PEDERSON, N. Y. Agr. Expt. Sta., Geneva. Jour. Bact., *53,* 4: 407-415. April, 1947.

Lactobacillus spp. isolated from milk and milk products usually are considered to be strains of *L. casei* (Orla-Jensen) Holland. These form smooth colonies and dextrorotatory lactic acid. In the present study a number of cultures isolated as rough or filamentous colonies from various parts of the human body, and usually considered as strains of *L. acidophilus* (Moro) Holland, have changed their characters over a period of years and now are identical with cultures of *L. casei*. It is concluded that *L. acidophilus* and *L. casei* are rough and smooth strains of a single type. D.P.G.

443. An Occurrence of Udder Infections Caused by a Thermoduric Streptococcus (Abs.). J. S. Bryan, Walker-Gordon Laboratory Co., Plainsboro, N. J. Jour. Bact., *53*, 4: 505. April, 1947.

Thermoduric streptococcus infection of the udders of cows caused an increased bacterial count of high-quality pasteurized milk. Milk from infected udders contaminated the equipment. This contamination was eliminated in routine cleaning and sterilizing after infected cows were located and removed from the supply. Detection of infected cows was difficult because the symptoms and physical appearance of the milk were not the same in each case. Laboratory pasteurization of the milk from each cow was necessary to confirm physical findings and to locate infected cows.

The thermoduric streptococcus apparently does not normally reside in the udder. These bacteria evidently are not important in milk from a public health standpoint but are important to the plant since they affect the pasteurized milk count. D.P.G.

444. The Significance of, and Methods for, Maintaining Moisture in Bacteriological Culture Media (Abs.). G. H. Chapman, Clinical Research Laboratory, N. Y. Jour. Bact., *53*, 4: 504. April, 1947.

An agar plate stored in an electric refrigerator loses an average of 0.1 ml. of moisture each day. This is detrimental to microbic growth. Moisture loss can be reduced by storing plates in metal cylinders and sealing the joint between top and bottom sections with waterproof adhesive tape, parafilm, or a rubber band. The vent hole also should be sealed. Tubes of culture media can be sealed with parafilm. D.P.G.

445. Some Observations on a Pleomorphic Bacillus Isolated from Different Lots of Processed Whole Milk Products (Abs.). Helen Z. Knight and E. H. Browne, M & R Dietetic Laboratories, Inc., Columbus, Ohio. Jour. Bact., *53*, 4: 501. April, 1947.

The incompletely identified bacillus appeared as long rods in chains, rods with central and plectoral globi, and dumbbell forms. A life cycle was observed beginning with long rods and ending with clumps of globi. Increasing nitrate content of the medium resulted in formation of filaments resembling mycelium. On blood agar the bacillus was a non-hemolytic, small, plump rod. The variations were non-permanent, the bacillus reverting to its original forms when transferred to standard milk agar. The presence of the bacillus had no effect upon the milk product. D.P.G.

CHEESE

446. The Effects of Lactobacilli on the Quality of Cheddar Cheese Made from Pasteurized Milk (Abs.). R. P. TITTSLER, G. P. SANDERS, H. E. WALTER, DONNA S. GEIB, O. S. SAGER, AND H. R. LOCHRY, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Dept. Agr., Washington, D. C. Jour. Bact., *54,* 2: 276. Aug., 1947.

Lactic starter was added to one lot of cheese as a control and lactic starter and a supplemental starter containing lactobacilli were added to each of the other lots. *Lactobacillus casei* (3 types), *L. arabinosus, L. pentosus, L. fermenti,* and *L. plantarum* grew rapidly in the cheese. From 50 to 500 millions per g. were present at from 2 to 12 weeks, depending on species, numbers added, and temperature of ripening (50 to 60° F.). *L. fermenti* produced gas and objectional flavor. *L. casei* increased acidity but not proteolysis; it increased flavor of "acid" nature and "short" body. Some strains of *L. arabinosus, L. pentosus,* and *L. plantarum* increased flavor and did not produce objectionable acidity. Other strains had little effect on quality.

L. bulgaricus, L. helveticus, L. lactis, and *L. acidophilus* were not detected after 2 weeks at either temperature, and the cheese grades were the same as those of the corrsponding controls. D.P.G.

CHEMISTRY

447. Effect of Dry Heat on Proteins. D. K. MECHAM AND H. S. OLCOTT. Western Regional Research Laboratory, U. S. Dept. Agr., Albany, Calif. Indus. and Engin. Chem., *39,* 8: 1023–1027. Aug., 1947.

Casein and other proteins were heated to 110–203° C. for 18 hr. in boiling, inert hydrocarbons. Solubility decreased with increasing temperatures up to 153° C., but there was no change in total *N* and little change in amide *N*. Above 153° C. extensive degradation occurred. B.H.W.

448. Process for Hardening Protein Fibre. F. C. ATWOOD. (Assigned to Natl. Dairy Products Corp.) U. S. Patent 2,428,603, Oct. 7, 1947 (15 claims). U. S. Patent Office Off. Gaz., *603,* 1: 128. 1947.

A relatively pure protein, such as casein, is dispersed in an alkaline solution and forced through an orifice into an acid-coagulating bath to form a continuous thread which then is stretched and finally hardened by means of a formaldehyde bath maintained between pH 4.5 and 7.0. R.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

449. Plastic Cream—Its Advantages in Ice Cream Making. A. C. DINGWALL, Dairy Consultant, New York City. Ice Cream Field, *50,* 2: 33, 74. Aug., 1947.

Because of the decreased volume accompanying the use of plastic cream instead of frozen 40% cream, considerable saving in transportation and storage costs results.

The following procedure was used successfully in one plant. Cream testing 50% fat first was separated from fresh milk. The cream then was pasteurized at 170° F. for 30 min. After cooling to 130° F., it again was run through a separator to produce cream testing 80% fat. Then it was forced through an internal tubular cooler by means of a worm-drive, which cooled the cream to 60° F. Cans filled with the cream were frozen and stored in a sharp room maintained at −20° F. W.C.C.

DISEASES

450. Brucellosis. Editorial. Amer. Med. Assoc. Jour., *134,* 10: 976. July 5, 1947.

Brucellosis still is erroneously believed to be a regressive, rare, and self-limited disease. Infection of susceptible persons will continue as long as contaminated food is ingested or there is contact with infected cattle, goats, swine, sheep, or horses. Only the relatively severe acute illnesses are diagnosed and reported. Chronic infections outnumber the acute by at least 10 to 1, but chronic infections rarely are diagnosed.

Mortality at present usually is low, but higher rates may occur in the presence of epidemics of the more virulent *suis* infection carried by raw milk. Cross-infection apparently is increasing; thus the hog or the cow may transmit *melitensis* or *suis* to man. Huddleson has estimated the following annual losses for Michigan alone, all from an estimated 108,000 infected cows: 222,904,000 lb. of market milk or enough to supply 557,000 persons for 1 year; 11,140,200 lb. of butter, enough to supply 655,300 persons for 1 year; 16,240 calves, representing 1,299,200 lb. of veal or 6,494,000 lb. of beef or a proportionate amount of dairy products if the calves were raised as milk-producing animals. On one farm 82% of the expected pig crop, or 54,180 lb. of dressed pork, was lost through infection.

Detailed discussion is given to failure to diagnose human chronic cases. The writer deplores the defeatist attitude toward treatment and calls for increasing "brucellosis-mindedness" and continued investigation. He concludes: "Pasteurization of all dairy products must remain the greatest bulwark against human infection until methods of control can be perfected." D.P.G.

451. Brucellosis in the United States. Current Comment. Amer. Med. Assoc. Jour., *134,* 9: 786. June 28, 1947.

The approximately 4,000 cases of human brucellosis reported annually to state health departments indicate that there are at least 30,000 to 40,000 actual cases. A method of laboratory diagnosis is needed that will reveal milder forms of the disease. A therapeutic agent is needed for the control of brucellosis in animals. The development of better methods of control, diagnosis, and treatment is one of the most pressing problems facing the medical profession. D.P.G.

452. Permeability of the Lactating Bovine Mammary Gland to Sulfonamides (Abs.). V. T. SCHUHARDT, T. B. CARROLL, L. J. RODE, AND HELEN LACY. The Brucellosis Research Project of the Clayton Foundation and the Univ. of Texas. Jour. Bact., *53*, 3: 367. March, 1947.

Sulfanilamide, sulfathiazole, sulfadiazine, sulfamerazine, sulfapyrazine, 2-sulfanilamido-5-bromo pyrimidine, and 2-sulfanilamido-5-chloro pyrimidine were administered orally to 2 cows. Sulfapyridine was administered to 4 cows. Each cow received 90 g. of the appropriate drug in 3 equal doses at intervals of 4 hr. Blood and milk samples were collected from each cow at 6, 10, 22, 34, and 46 hr. after the initial drug dose. These were tested for free sulfonamide concentrations. The last 3 samples of both blood and milk were tested for total sulfonamide concentrations.

Sulfathiazole, sulfapyrazine, and sulfadiazine attained maximum blood levels of less than 5 mg.% of the free drug. Only the latter was demonstrable in the milk, attaining a concentration of 1.1 mg.%. All the other drugs attained maximum blood levels ranging from 5 to 13 mg.% free drug. Only sulfanilamide and sulfapyridine came through the mammary gland in concentrations approaching those found in the blood. Sulfapyridine reached higher levels in both blood and milk. D.P.G.

453. The Use of Sodium Sulfamerazine in Foot Rot (Infectious Pododermatis, Interdigital Phlegmon) in Cattle. F. T. CANALIN. Amer. Vet. Med. Assoc. Jour., *111*, 847: 278–281. Oct., 1947.

Sodium sulfamerazine was injected intravenously into 34 cows and 1 bull as a cure for foot rot. In all but one case, recovery was rapid, complete and satisfactory. Cows with secondary infections involving the deeper structures of the foot may require 4–6 days for recovery; other cases require only 2–3 days. Optimal dosage is approximately 3 g. of sulfamerazine per 100 lb. of body weight and should be given in a single dose. No toxic results were noted. T.M.L.

FEEDS AND FEEDING

454. Handling Fresh Alfalfa before Dehydration. R. E. SILKER AND H. H. KING, Kansas Agr. Expt. Sta., Manhattan. Indus. and Engin. Chem., *39*, 9: 1163–1165. Sept., 1947.

Dehydrated alfalfa meal obtained from field-chopped alfalfa contained slightly less carotene than did meal produced in the conventional manner from long alfalfa, which was chopped just prior to dehydration. Before dehydration, the field-chopped hay lost about 3% of its carotene per hour, but it lost less carotene during dehydration than did the long hay. Field chopping apparently does not seriously reduce the carotene content of alfalfa meal. B.H.W.

455. Vapor Pressure of Water Adsorbed on Dehydrated Alfalfa. W. G. Schrenk and H. H. King, Kansas Agr. Expt. Sta., Manhattan. Indus. and Engin. Chem., *39,* 9: 1165–1167. Sept., 1947.

The vapor pressure of water adsorbed on dehydrated alfalfa was measured in the region of 2.2 to 14.1% moisture and in the temperature range from 17 to 50° C. Typical S-shaped adsorption isotherms were obtained. Protein content of the meal had little effect on vapor pressure. Blanching prior to dehydration did not alter the position or shape of the vapor pressure curves. B.H.W.

456. Storage of Commercial Dehydrated Alfalfa Meal. R. E. Silker, W. G. Schrenk, and H. H. King, Kansas Agr. Expt. Sta., Manhattan. Indus. and Engin. Chem., *39,* 9: 1160–1162. Sept., 1947.

Alfalfa meal held in storage cave at about 38° F. and 82% relative humidity showed improved carotene stability at low-temperature storage and when the moisture content of the meal was held higher than its moisture at the time of milling. No advantage was derived from the use of waterproof packaging for meal storage. B.H.W.

FOOD VALUE OF DAIRY PRODUCTS

457. De Voedingswaarde van Melk, Vergelijking van Koemelk en Vrouwenmelk (Nutritive Value of Milk, Comparison between Cow's Milk and Human Milk). Chr. Engel, Central Instituut voor Voedingsonderzoek, Utrecht, the Netherlands. Netherlands Milk and Dairy Jour., *1,* 1: 43–56. 1947.

A compilation of recent literature dealing with composition of cow's and human milk and colostrum, including tables on essential amino acids, fatty acids, vitamins, and minerals, is presented. Comparisons between the two types of milk are made wherever possible. 59 references. I.P.

ICE CREAM

458. Effect of Different Homogenizer Valves on Some Abnormal Ice Cream Mixes. A. Leighton, Division of Dairy Research Laboratories, Bureau of Dairy Industry, Washington, D. C. Ice Cream Rev., *31,* 2: 42. Sept., 1947.

Mixes which were difficult to whip when processed in the conventional manner were found to whip easier with a change in homogenization procedure. Comparative trials in which portions of the same mix were homogenized with an old first-stage valve, a new first-stage valve, and with the second-stage valve operated at pressures normally used with the first-stage valve revealed that best results were obtained when the mix was processed with the second-stage valve only. It further was shown that the portion of the mix processed with the old first-stage valve produced a mix of better whipping ability than when the new first-stage valve was employed. The use of a multi-flow homogenizer likewise produced a mix with better whipping properties than when processed with either of the first-stage valves. The use of a high pressure on the second-stage valve not only improved the whipping ability of the mix but also made the mix thinner, so that it handled better on the cooler, reduced the motor load in freezing, and produced better ice cream with respect to its body and texture.

When mixes of low whipping ability containing 8, 12, and 16% fat were used, the improvement in whipping ability through the use of the second-stage valve was most pronounced with the higher fat mixes. The whipping ability of chocolate mixes, as well as mixes prepared from butter, was improved by processing with the second-stage valve only. With mixes of normal whipping ability, there was little or no difference in the behavior of mixes treated with the first-stage as compared with that processed with the second-stage valve only. In another trial with a normal mix, better results were obtained with the first-stage valve than when the mix was processed with the second-stage valve.

No explanation for differences in the whipping ability of these mixes could be found from a microscopic examination of them. On the basis of the Farral index, all mixes showed satisfactory breakup of the fat globules. One explanation for the differences observed is that in some way the adsorption of protein on the butterfat globule was more thorough in mixes when processed with the second-stage valve only. Results obbtained in this investigation indicate the need for a better way of expressing homogenization efficiency. W.J.C.

459. Mix Stabilizers and Whipping Agents. P. H. Tracy, Univ. of Illinois. Ice Cream Rev., *31,* 2: 80. Sept., 1947.

The origin, method of manufacture, and the possible advantages and disadvantages for use in frozen dairy foods of a number of stabilizing agents, including gelatin, carob bean, pectin, dariloid, Irish moss, quince-seed extract, gums, and sodium carboxymethyl cellulose, are discussed.

The general nature and properties of several emulsifying agents being offered to the ice cream industry are presented. These emulsifying agents serve much the same function as egg yolk in ice cream. Their use will result in faster whipping, smaller air cells, a drier ice cream, and a smoother texture. One possible disadvantage of using emulsifying agents in ice cream is that they may cause shrinkage. W.J.C.

460. The Case for Liquid Sugar. C. F. KOERVER, The Borden Co., N. Y. Ice Cream Field, *50*, 2: 16–18, 67, 77. Aug., 1947.

The following specifications are given for a good liquid sugar-sucrose: Color, white and crystalline clear; sugar, sucrose 99.5% and invert 0.5% of total sugar; ash content, 0.1% of total solids; brix, not higher than 67.5; pH 6.3; bacteria, less than 1,000 per ml.; *B. coli*, negative. Taking the sirup density with a brix hydrometer calibrated to 68° F. after diluting the sirups to twice their weight with water is recommended.

Ice cream plants in metropolitan areas or communities in which refineries are located profitably may use liquid sugar provided their annual minimum sugar consumption is 300,000 lb. In communities away from these areas, a minimum of 500,000 lb. annually would be required to make the necessary installations profitable. Delivery in drums is uneconomical, but delivery by tank trucks of 1,000 to 2,500 gallon capacity or in tank cars of 6,000 to 8,000 gallon capacity is considered good practice. The pipeline from the street level to the storage tanks should be at least 2.5 in. in inside diameter and may be of galvanized iron.

Protection from moisture and the use of "Sterilamps" in storage tanks are recommended. Accurate weighing of sirup delivered is imperative and the use of weighing tank scales for all liquids going into the pasteurizer is recommended.

Iron in liquid sugar is objectionable because it will cause undesirable discoloration in chocolate ice cream and may contribute to oxidized flavor in general. The claim is made that invert sugar in liquid sugar to the extent of 15% of the total sugar content is satisfactory for ice cream manufacture, but texture difficulties may result if this value is exceeded. The amount of invert sugar can be increased to 20 or 25% in the case of ices and sherbets. Mention also is made of using a blend of liquid sugar (sucrose) and corn sirup in the ratio best suited for plant needs. For miscellaneous purposes it is well to have a small amount of dry sugar on hand. W.C.C.

461. Evaluation of Ice Cream Sweeteners. H. A. BENDIXEN, Washington State College, Pullman. Ice Cream Field, *50*, 2: 20–22. Aug., 1947.

A classification and brief description is given of the sweeteners commonly used in ice cream. Literature is cited to show that the concentration at which the sweetness of sweeteners is evaluated materially influences the results. When corn sugars are used in combination with sucrose they seem sweeter than when tasted alone.

It is stated: "For each pound of cane sugar to be replaced about 1.0–1.1 pounds of dextrose, 1.3–1.5 pounds of high conversion corn sirup, 1.7–2.0 pounds corn sirup solids, 2.5 pounds of regular sirup, 1 pound of invert sirup, 1.3–1.4 pounds of honey should be used."

Several methods of inverting sugar, as reported in the literature, are cited, and brief mention is made of the use of honey as a sweetener.

W.C.C.

462. Corn Is Also Ice Cream Sweetener. R. J. TREBILCOCK, Corn Products Sales Co. Ice Cream Field, *50,* 2: 24. Aug., 1947.

The author claims that best results are obtained in sweetening ice cream, ices, and sherbets by using two or more sweeteners. A combination of ⅕ dextrose, ⅕ corn sirup, and ⅗ cane or beet sugar is recommended. W.C.C.

463. Why and How to Sell Ice Cream in a Grocery Store. J. M. CUMMINGS, General Ice Cream Corp. Ice Cream Field, *50,* 2: 28, 30, 32. Sept., 1947.

Reference is made to a survey by the Homemakers Guild of America in which it was reported that 77.1% of the women interviewed preferred to buy all their food and household needs in one store but only 3.6% were buying their "take-home" ice cream in the one-stop super markets. An additional 22½% were buying their "take-home" ice cream in smaller neighborhood grocery stores, and 79.3% of the carry-out ice cream was being sold in non-grocery outlets.

Sales of ice cream in grocery-type stores are on the increase, as the author's company sold 22% of its ice cream to grocery stores in 1945, 23% in 1946, and an even larger percentage in 1947.

The value of point-of-sale advertising and suitable display cabinets properly located is stressed for selling ice cream. Insulated bags and, in some cases, dry ice in addition, are considered helps in selling ice cream in the grocery store. Proper pricing of the ice cream and maintaining quality are essential to successful selling. W.C.C.

MILK

464. The Practical Significance of So-called Heat-resistant Coliform Organisms in the Coliform Testing of Pasteurized Milk (Abs.). EDYTHE C. ALFF AND L. BUCHBINDER, Bureau of Laboratories, New York City Dept. of Health. Jour. Bact., *53,* 4: 505. April, 1947.

Heat-resistant coliforms rarely, if ever, were found in the final product in a milk-plant study of raw, line, and final milk. A study of 1,000 samples of raw milk over a period of 1 year showed that far fewer coliform organisms are found in raw milk than commonly are used in the laboratory pasteurization of coliforms. When reasonable numbers of test organisms were used, complete kill always was obtained when employing the technic of complete submersion, but not when two technics for partial submersion were used. It is concluded that coliforms are not found after laboratory pasteurization when the proper method and reasonable numbers of organisms are used. Heat-resistant coliforms seem to have no practical significance in the coliform testing of pasteurized milk. D.P.G.

465. Method and Application for Deaerating Liquid Foods. P. F. SHARP, D. B. HAND, AND E. S. GUTHRIE. (Assigned to Cornell Research Foundation.) U. S. Patent 2,428,044, Sept. 30, 1947 (13 claims). U. S. Patent Office Off. Gaz., *602*, 5: 737. 1947.

The vitamin C content is preserved and the development of certain off flavors retarded by deaerating milk prior to bottling under air-free conditions. The authors describe a continuous method of deaeration by spraying the milk at a temperature of 105–115° F. into a vacuum chamber where an amount of water is evaporated equal to about 0.5 to 2% of the weight of the milk and the temperature is reduced by about 7 to 15° F. The deaerated milk is removed continuously from the bottom of the chamber at approximately the rate at which it enters and is then cooled and packaged under subatmospheric pressure to prevent aeration. A regenerative heating and cooling step may be employed for greater economy. R.W.

466. Liquid Treating Apparatus. P. F. SHARP, D. B. HAND, AND E. S. GUTHRIE. (Assigned to Cornell Research Foundation.) U. S. Patent 2,428,045, Sept. 30, 1947 (8 claims). U. S. Patent Office Off. Gaz., *602*, 5: 737–738. 1947.

An apparatus in which liquids, such as milk, may be deaerated or gaseous or readily vaporizable materials may be removed continuously is described. The product is introduced as a spray through a perforated pipe into a cylindrical tank maintained under vacuum. A definite volume of gas-free liquid is allowed to collect in the bottom of the tank and then is removed continuously by means of a pump from an outlet at the bottom. A leveling device controls the rate of intake and the volume of deaerated liquid maintained in the chamber. R.W.

467. Flavors in Milk from the Farm. F. J. DOAN, Dept. of Dairy Husbandry, Pennsylvania State College. Milk Dealer, *36*, 11: 72–76. Aug., 1947.

Undesirable flavors found in milk coming from the farm, listed in the order of their frequency, are: (a) feed and weed, (b) cowy or barny, (c) salty, (d) rancid, (e) bacterial, (f) flat, (g) bitter, and (h) foreign. The cause and prevention of these undesirable flavors are discussed. C.J.B.

468. **Quality Milk Production Program.** ANONYMOUS. Milk Dealer, *36,* 11: 47, 48, 80. Aug., 1947.

The Dixie Dairy Co. of Gary, Indiana, has made effective use of the Indiana State champion 4–H Club demonstration team to help sell the company's producers on quality milk production. The team is used at farm group meetings. The program for the meetings and the team script are given. C.J.B.

469. **Cooling Jacket for Milk Cans.** C. P. VAN FRANK. U. S. Patent 2,426,475, Aug. 26, 1947 (2 claims). U. S. Patent Office Off. Gaz., *601,* 4: 663. 1947.

Milk or cream in the conventional type of can is kept cold by means of a jacket which fits loosely over the can and is sealed to a base plate on which the can rests. Cold water is admitted into the space between the can and semiporous jacket. R.W.

470. **Milk Strainer Pad Dispenser.** R. SCHNEIDER. U. S. Patent 2,428,530, Oct. 7, 1947 (2 claims). U. S. Patent Office Off. Gaz., *603,* 1: 108. 1947.

To keep milk strainer pads clean and protected a dispenser has been developed comprising a covered cylindrical storage receptacle with a hinged bottom door through which the pads are easily removed one at a time as required. R.W.

471. **Cooperative Advertising Pays.** H. WAGENSEIL, Hugo Wagenseil and Associates, Dayton, Ohio. Milk Dealer, *36,* 11: 41, 42, 100, 101. Aug., 1947.

A description of the cooperative advertising and promotion plan developed in 1938 by milk dealers in Dayton, Ohio, to move surplus milk is given. The record of accomplishments is worthy of the attention of dealers in other cities. C.J.B.

472. **Cream Separator.** L. D. MCNEILL. U. S. Patent 2,428,429, Oct. 7, 1947 (1 claim). U. S. Patent Office Off. Gaz., *603,* 1: 84. 1947.

To remove cream from the top of a bottle of milk there is submerged into the cream layer a device consisting of a cylinder having a flange and gasket for sealing the top of the device to the cap seat of the bottle and a perforated bottom which permits most of the cream to be poured off without mixing with the lower layers of low-fat milk. R.W.

SANITATION AND CLEANSING

473. The "Reversal", Neutralization, and Selectivity of Germicidal Cationic Detergents. M. Klein and Zelma G. Kardon, Dept. of Bacteriology, School of Medicine, Univ. of Pennsylvania, Philadelphia. Jour. Bact., *54,* 2: 245–251. Aug., 1947.

Careful distinction is made between reversal of the bacteriostatic activity of cationic detergents after contact with bacteria and neutralization of activity before contact. Anionic detergents tested did not reverse the activity of two cationic detergents. However, in one instance, bacteriostatic action of zephiran against Gram-negative bacteria was neutralized, while the same action against Gram-positive bacteria was not neutralized. Zephiran has a high degree of selectivity and possesses several hundred-fold greater activity against Gram-positive than against Gram-negative bacteria. D.P.G.

474. Inactivation of the Germicidal Action of Quaternary Ammonium Compounds (Abs.). C. A. Lawrence, Winthrop Chemical Co., Inc., Renssalaer, N. Y. Jour. Bact., *53,* 3: 375. March, 1947.

A suitable inactivator for the antibacterial action of quaternary ammonium germicides must meet certain requirements. It should, as completely as possible, neutralize the bacteriostatic and bactericidal actions of the quaternaries and maintain the neutralized state for a prolonged period of time. The neutralizing agent itself should not possess antibacterial properties. Several anionic detergents, including soaps, do not meet these requirements. A series of compounds that give a precipitate in the presence of quaternary ammonium solutions failed to inactivate completely the germicidal action of the latter. A sulfonic acid derivative, the sodium salt of symmetric bis (*m*-amino-benzoyl-*m*-amino-*p*-methylbenzoyl-1-naphthyl-amino-4,6,8-trisulfonic acid) carbamide, closely meets the requirements for an inactivator. D.P.G.

MISCELLANEOUS

475. Soil Conservation. Anonymous. Milk Dealer, *36,* 11: 43–44, 92–94. Aug., 1947.

The importance of soil conservation to the dairy industry is discussed and methods are set forth whereby milk dealers and other businessmen can support the national program. C.J.B.

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